PREVALENCE AND CLINICAL ASSOCIATIONS OF ANTI-C1Q ANTIBODIES IN PEDIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

A dissertation submitted in partial fulfillment of the requirements for the award of M.D. Pediatrics degree of The Tamil Nadu Dr. MGR Medical University, Chennai, to be held in May 2018

CERTIFICATE

This is to certify that the dissertation titled **"Prevalence and clinical association of anti C1q antibodies in pediatric SLE"** is the bonafide, original work done by **Dr. George Ipe Vettiyil,** under my guidance, during his academic term from **April 2016** to **March 2018**, at the Christian Medical College, Vellore, in partial fulfillment of the rules and regulations of The Tamil Nadu Dr. MGR Medical University, Chennai, for the award of the degree of MD Paediatrics.

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ABSTRACT

Background:

Anti-C1q has been associated with systemic lupus erythematosus (SLE) as well as in other connective tissue diseases. They have been considered as a marker for disease activity and presence of nephritis in previous studies

Objectives:

Aim of this study was to determine the prevalence of anti- C1q antibodies in the pediatric SLE population and to determine clinical associations of elevated anti- C1q antibody levels especially with lupus nephritis.

Methods:

Sera of 150 pediatric SLE patients who fulfilled ACR criteria for SLE were recruited. After obtaining informed consent, blood samples were tested for anti- C1q antibody by commercially available ELISA kit. Prevalence of anti-C1q and its association with lupus nephritis were determined. .

Results:

Out of total 150 children with SLE, anti- C1q positivity was present in 95 children (64%), at a cut off value of 20U/ml. Children with proteinuria, low C3, low C4 and anti dsDNA positivity had were significantly more likely to have anti- C1q antibody positivity. Children with lupus nephritis were significantly more likely to have anti C1q antibodies

positive than children without renal involvement (74% vs. 51% , p= 0.02). Among the children with lupus nephritis, children with active renal disease were more likely to have anti- C1q positivity than in children with quiescent disease (88% vs. 53% , p= 0.002). Anti-C1q antibodies had a sensitivity of 74% and specificity of 54% at a cut off value of 22U/L , for renal disease in pSLE

Conclusion:

Out study confirms previous findings of the association of anti-C1q antibodies with nephritis and disease activity in pSLE. Anti-C1q antibody titers were found to have positive correlation with renal disease in children with pediatric SLE, and could be used as an adjunctive biomarker in monitoring disease activity in children with lupus nephritis

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Key words: Systemic lupus erythematosus; anti-C1q antibodies; nephritis

INTRODUCTION

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic, autoimmune disease characterized by multi-system inflammation and the production of autoantibodies directed against self-antigens. SLE is described as one of the great mimics in medicine, as symptoms attributed to the disease process are myriad and can involve any organ system in the body. Diagnosis of the disease requires a high degree of suspicion. Compared to adults, children with SLE have more severe disease process and greater organ involvement. Renal disease has been found to be commoner in pediatric SLE as compared to adults.

Complements play a very important role in our body and are one of the first lines of defense against pathogenic micro-organisms. There are three complement pathways which act via activation of numerous complement components. The final common pathway leads to formation of a membrane attack complex, which helps in opsonization of bacteria.

Deficiency of early complement components are genetically associated with development of SLE.(1) Individuals with deficiency of C1q, the first component of the classical complement pathway, have the highest prevalence of SLE and the most severe disease manifestations. The strongest association is seen in patients with homozygous C1q deficiency, of whom 88% developed SLE and 30% developed glomerulonephritis.(2) In vitro, physiological concentrations of C1q inhibit interferon alpha production, by plasmacytoid dendritic cells stimulated with nucleic acid containing immune complexes, suggesting a regulatory effect of C1q in response to immune complexes. It has also been

shown to help in the clearance of immune complexes. In patients with SLE, C1q levels were reduced in glomerulonephritis flares.(3) In patients with renal lupus, presence of C1q at the time of renal biopsy was associated with worse renal outcomes.(4) Acquired antibodies against the collagen like region of C1q were present in the glomerular basement membrane of patients with proliferative lupus nephritis, at a much higher concentration than in the serum, suggesting a role for this antibody in the pathogenesis of lupus nephritis.(5) C1q were aggregated within immunoglobulin G in renal sub-endothelial deposits in active proliferative lupus nephritis, as seen on electron microscopy, further supporting a pathogenic role of anti-C1q.(5) In another study, an increase in anti-C1q level preceded renal flare by 2.3 months and was more specific for renal flare than increase in anti- dsDNA levels.(6) The presence of anti c1q at the time off renal biopsy was associated with worse renal outcome by the ACR renal response criteria. Patients with active lupus nephritis had a higher prevalence of anti c1q than those without lupus nephritis, 74% vs. 32% ($p<0.0001$).(7) Anti-C1q increased almost 6 months prior to renal involvement in 50% of patients with SLE (8) and were associated with proliferative forms of lupus nephritis.(9) Anti-C1q concentration correlated with activity on modified SELENA SLEDAI and the SLICC renal activity score.(10) With immunosuppressive therapy for membranoproliferative lupus nephritis with either cyclophosphamide or azathioprine, anti-C1q disappeared by week 12 and remained undetectable throughout one year of follow up.(11) Several studies have proved that anti-C1q antibody is superior to other serological markers in identifying a flare of lupus nephritis.

FQ Wu and colleagues investigated 90 pediatric SLE patients, 43 in active stage and 47 in remission. They demonstrated significantly higher levels of anti-C1q antibodies in children with active disease which had a positive correlation with SLE disease activity index (SLEDAI) score. The sensitivity of C1q antibody in pediatric SLE was sound to be 95.6% and specificity was 97.5%. (12)

Renal disease is the most dreaded complication of SLE and is the main cause of mortality in children. Renal biopsy is the current gold standard to diagnosed lupus nephritis. This procedure is invasive, painful and requires in-hospital care. A biomarker with high sensitivity and specificity for lupus nephritis, which can predict nephritis or flare much before the onset or renal function abnormalities or histopathological changes can help avoid unnecessary invasive procedures in children.

In one of the few studies on pediatric lupus nephritis, Mohammed Kader and colleagues (13), evaluated levels of anti-C1q antibodies in children and adolescents, after classifying them into three groups- group 1 with SLE and active lupus nephritis, group 2 with SLE and without active lupus nephritis, group 3 with healthy controls. They found significantly higher levels of these antibodies in children with active lupus nephritis and those without and healthy controls. They concluded that anti-C1q antibodies can be used as a considerable marker for lupus nephritis in the pediatric population with 97.5% sensitivity and 65% specificity with a cut off value of 12U/L. (13)

As detailed above, evidence suggests that anti-C1q is associated, not only with lupus nephritis, but also with lupus nephritis flares and response to treatment. Studies from

India on the efficacy of anti-C1q are few and far in between. In fact there is no published trial evaluating the role of anti-C1q in Indian children with SLE. Hence this study is being undertaken with an aim to evaluate and understand the role of this antibody as a marker for renal injury in pediatric SLE and also to elucidate its association with other clinical manifestations of SLE, such as cutaneous lupus.

AIMS AND OBJECTIVES

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AIMS

- To evaluate the prevalence of anti-C1q antibody in pediatric SLE
- To determine its clinical correlation with disease manifestations in pediatric SLE

OBJECTIVES

1. To determine the prevalence of anti- C1q antibodies in the pediatric SLE population presenting to pediatric rheumatology OPD
2. To determine clinical associations of elevated anti- C1q antibody levels
3. To define the association of anti- C1q with renal involvement in pediatric SLE and to determine the sensitivity and specificity of anti-C1q antibodies in pediatric lupus nephritis.
4. To assess correlation of elevated anti-C1q antibody levels with SLEDAI score.

LITERATURE REVIEW

LITERATURE REVIEW

INTRODUCTION

Systemic lupus erythematosus (SLE) is a heterogeneous, chronic autoimmune disease characterized by multi-system inflammation and the production of antibodies directed against self- antigens. It can cause significant morbidity and mortality and can occur at any age, though it is most frequent in women of child–bearing age. Childhood onset SLE constitutes about 15 – 20% of all cases of SLE. Although, its etiopathogenesis, clinical features and laboratory abnormalities are similar to adult onset SLE, children have more severe disease process. They also have problems with physical and emotional growth, drug toxicities and life-long burden of chronic disease. Renal disease is commoner in children and is the most common cause of morbidity and mortality in pediatric SLE. SLE as a disease process was described in the 13^{th} century; however diagnosis of SLE is a field of active research even today.

Complements play a very important role in the human body. They are components of both innate and adaptive immunity and help in the opsonization of pathogens. They are one of the first lines of defense against infection in humans. There are three complement pathways of equal importance and C1q is the initiation molecule of the classical complement pathway.

Genetic deficiency of early complement components has been classically associated with development of SLE like clinical features. Studies have shown that in patients with

homozygous C1q deficiency, 88% developed SLE and 30% developed lupus nephritis. SLE is an autoimmune process characterized by formation of autoantibodies against self-antigens and studies in the last decade have demonstrated the presence of antibodies against C1q in patients with SLE. These antibodies are not specific for SLE and were initially described in hypocomplementemic urticarial vasculitis. Other diseases where anti-C1q antibodies are described are mixed connective tissue disorders, Felty syndrome, hypocomplementemic urticarial vasculitis syndrome and SLE. These antibodies are also described in healthy people with population prevalence described between 2 – 8%.(14,15) However the role of these antibodies and association with clinical disease have been best described in SLE, especially lupus nephritis. Several studies have proven that anti-C1q is superior to other serological markers in identifying a flare of lupus nephritis.(16)

There are various studies which have demonstrated that higher titers of anti-C1q correlated with active SLE disease. Anti-C1q also showed a positive correlation with SLEDAI score, which is one of the disease activity scores used for both children and adults. The sensitivity of C1Q antibody in pediatric SLE was found to be 95.6% and specificity was 97.5%.(12)

Renal disease is the most dreaded complication of SLE and is the main cause of mortality in children. Renal biopsy is the current gold standard to diagnose lupus nephritis. This procedure is invasive, painful, and expensive and requires in-hospital care. A biomarker with a high sensitivity and specificity for lupus nephritis, which can predict nephritis or flare much before the onset of proteinuria, renal function abnormalities or histopathological changes can help avoid unnecessary invasive procedures in children.

Mohammed Kader and colleagues evaluated levels of anti-C1q antibodies in Egyptian children and adolescents, after classifying them into three groups- Group 1 with SLE and active lupus nephritis, Group 2 with SLE and without active lupus nephritis, Group 3 with healthy controls. They found significantly higher levels of these antibodies in children with active lupus nephritis than those without and healthy controls, with a median (range) of [27.5(14-83), 9(2.5-30), 7 (2-13)], respectively.(13) They concluded that anti-C1q antibodies can be used as a considerable marker for lupus nephritis in the pediatric population with 97.5% sensitivity and 65% specificity with a cut-off value of 12U/L.(13) Anti-C1q antibodies were found to rise almost 6 months prior to the onset of proteinuria and renal function abnormalities and were higher titers was found in patients with proliferative lupus nephritis.

Hence, anti-C1q antibodies seem to be a promising new biomarker for diagnosing active lupus nephritis and for predicting disease flare. However, most studies on this novel biomarker are done in the adult population. Studies from India evaluating the efficacy of anti-C1q are few and far in between. In fact there is no published trial evaluating the role of anti-C1Q in Indian children. Hence this study is being undertaken with an aim to evaluate and understand the role of this antibody as a biomarker in pediatric lupus nephritis.

HISTORY OF LUPUS

Lupus was first named in the 13th century by Rogerius, with the Latin word for wolf, as the cutaneous manifestations of the disease described looked similar to a wolf bite. Osler first described systemic manifestations of the disease without skin involvement. The history of lupus is divided into classical, neoclassical and modern periods. In the classical period(1230-1856), various physicians described the cutaneous manifestations of lupus, including malar rash and discoid lupus.(17) During the neoclassical period (1872 – 1948), Kaposi first described systemic manifestations of lupus. Kaposi proposed that there were two forms of lupus: Disseminated form and discoid form. In 1904, Osler in Baltimore and Jadassohn in Vienna established the existence of the systemic form of the disease. Autopsy studies over the next 30 years lead to the establishment of Libman Sacks endocarditis and wire loop lesions in lupus nephritis. This led Kemperer and colleagues to propose that SLE was a 'collagen vascular disease', a term which is still widely used more than 80 years later.(17)

1948 was an important year in the history of lupus with Hargraves and colleagues discovering LE cells, which marked the beginning of the modern era of Lupus. LE cells were found in the bone marrow of individuals with acute disseminated lupus and the investigators postulated that the cell is the result of phagocytosis of free nuclear material. This discovery ushered in the era of immunology in lupus research. This, along with discovery of the pharmacological uses of cortisone, changed the way lupus was viewed up until that point in time. Friou, applied the technique of indirect immunofluorescence to demonstrate anti-nuclear antibodies in individuals with lupus. Subsequently other auto-antibodies were

described. The familial occurrence of SLE was first described by Leonhardt in 1954 at the Johns Hopkins institute.

In the last decade, significant advances have been made in the diagnosis and treatment options for lupus. Prognosis is better now than it was in the last century with earlier recognition of disease, better diagnostic tests with higher sensitivity and specificity, drugs which control the disease better and the application of biologics to treat the disease.

Although, SLE is a chronic multisystem autoimmune disease affecting children and adults, manifestations of the disease and severity of organ involvement differs between children and adults. Children with SLE have more widespread organ involvement and more severe disease. They also have problems with physical and emotional growth and development, treatment toxicities and lifelong burden of chronic disease.(18)

EPIDEMIOLOGY:

Global burden of disease

Childhood onset SLE constitutes about 15-20% of all SLE cases(19). In absolute numbers, pediatric SLE is a rare disease, with an incidence reported of 0.3 – 0.9 per 100,000 children, and prevalence of 3.3 – 8.8 per 100,000 children, which is lower than the prevalence described in adults (20-70/100,000). A higher frequency of SLE is reported in African, Asian and Afro-American children.(20) These ethnic groups are also found to have more severe disease process. In most studies the median age at onset of SLE is 11 -12 years and the disease is quite rare in children younger than 5 years of age. There is an impressive female preponderance for the disease with more than 80% of children with SLE reported to be girls.(21) There is a 4:1 female : male ratio pre-puberty, with the ratio changing to 8:1 post puberty.(22)

Indian burden of disease

Screening of a north Indian population by Malaviya et al. in 1993 gave an SLE point prevalence of 3.2 per 100,000 population. This was a much lower figure than reported from the west, which varied from 12.5 per 100,000 adults in England to 124 per 100,000 adults in the USA. Although pediatric SLE is a rare disease, Habibi et al in 2011, presented data which found that 3.9% of pediatric rheumatology OPD attendees had SLE with 3.8:1 female: male

ratio.(14) There are no published studies from India evaluating population prevalence of pediatric SLE.

According to a study published from the National Institute of Immunohematology, in 2013, 84% of the study population with SLE was girls and the mean age at onset was 9.2 years (range 5-14 years). However in a retrospective chart review from a tertiary care center in Kerala, female : male ratio was found to be 2.3:1, which was much less than previously described.(23) Pediatric patients had more CNS involvement and lupus nephritis was present in 52% of children at the time of presentation(24). In a study conducted by Samanta et al, lupus was found to be three times more common in Indians as compared to Caucasians.

ETIOPATHOGENESIS:

The etiology of SLE is complex, multifactorial and polygenic. In a genetically susceptible individual, loss of the normal control over immunologic reactivity to antigens combined with environmental triggers leads to clinical manifestations of the disease.

SLE is caused by loss of self-tolerance, with development of antibodies against self-antigens, most commonly nucleic acids. These antibodies develop many years prior to the development of clinical features. Nucleic acid antigens are usually inaccessible to antibody producing cells and are cloistered within cells. During apoptosis these antigens are released. In patients with SLE there is either an excess of apoptosis or decrease in the debris clearing capacity.(25)Skin cells in patients with SLE are particularly susceptible to UV light and undergo early apoptosis in the presence of UV light. These cells also release their nucleic acid materials, which act as targets for antibody production. Genetic predisposition to SLE is found in individuals with congenital deficiencies of complement factors C1q, C2 and C4. In addition, certain HLA types such as HLA B8, DR2 and DR3 are found with greater frequency in patients with SLE than in the general population. Although, clearly a genetically mediated disease SLE occurrence in families is sporadic and its concordance is incomplete (2-5% among dizygotic twins and 25-60% among monozygotic twins).

Increased incidence of SLE in females especially in the reproductive years implies the role of hormones in the pathogenesis of the disease. Estrogen directly affects cytokine network and also increases the calcineurin mRNA levels in SLE T cells.

There are environmental influences, which produce epigenetic modifications (DNA methylation, modification of histone tails and non-coding RNAs) and these contribute to expression of SLE phenotype.(26) According to a report published by the National Institute of Environmental Health Sciences in 2010, occupational exposure to silica is the only definite environmental exposure, which predisposes to SLE.(27) Cigarette smoking is classified as 'likely' to cause autoimmune diseases and it is known that smoke exposure worsens flares in people with SLE.

Many organisms have been proposed as SLE triggers and these include bacteria, viruses and parasites. Notable among these is Ebstein Bar virus and it is only against this organism that some conclusive proof exists to prove its association with autoimmune diseases. Certain antigens in SLE, specifically Ro and La have been found to have similarity with EBV nuclear antigen 1. This is thought to initiate molecular mimicry, which is one of the explanations for the etiopathogenesis of SLE. In the matched case control study conducted by Parks et al, EBV IgA seroprevalence was strongly associated with development of SLE (OR 5.6, [95% CI] 3.0-10.6).(28) They also found that the strongest association was found in those above 50 years of age, and hypothesized that repeated EBV infection had a causal relationship with SLE. James et al conducted similar studies in children and adolescents and found that EBV exposure was associated with SLE in a significant proportion of children (odds ratio [OR] 49.9, 95% confidence interval (95% CI 9.3-1,025). (29)

Drugs can induce a lupus like syndrome and common drugs implicated include hydralazine, minocycline and some anticonvulsants. The clinical manifestations of drug induced lupus

resolve with the discontinuation of the offending drug. This clinical entity is associated with anti-histone antibodies.

Autoantibodies form circulating immune complexes and deposit on various tissues, leading to local complement activation, initiation of a pro-inflammatory cascade and tissue damage. Both innate and adaptive immunity have been implicated in the immune system dysregulation seen in SLE. There is an increase in the production of interferon α by plasmacytoid dendritic cells. This increases production of pro-inflammatory cytokines such as IL-1, IL-2, IL-6, IL-10 etc., T and B cell auto-reactivity and loss of self-tolerance which is seen in SLE. This type of cytokine profile is called as the Type 1 interferon signature and is seen in many patients with SLE. (25)

Both B and T cells have functional impairment in SLE. B cells have increased auto-reactivity, enhancing their ability to form auto-antibodies following exposure to self-antigens. Also cytokines such as B lymphocyte stimulator promotes abnormal B cell number and function. There is decrease in T regulatory cells, with increase in T memory cells. SLE T cells also demonstrate abnormal signaling and increased auto-reactivity and are resistant to attrition by normal apoptotic pathways. (25)

CLINICAL MANIFESTATIONS OF DISEASE

SLE is a multisystem disease which can affect any organ system, however skin manifestations; arthritis, neuropsychiatric problems and renal disease are the commonest presenting features. SLE is one of the great mimics, in that it can impersonate numerous diseases. In the absence of the typical malar rash, diagnosis of SLE can be delayed and requires a high degree of suspicion.

Non-specific constitutional symptoms

Fever, fatigue, weight loss, anorexia, arthralgia and alopecia can be the initial presenting symptoms of SLE in children. They can also present with generalized lymphadenopathy and hepatosplenomegaly.(21) Thus, SLE can be a differential diagnosis for numerous pediatric illnesses such as fever of unknown origin, lymphoreticular malignancies, other autoimmune conditions, infiltrative disorders such as Langerhans cell histiocytosis etc.

Cutaneous manifestations:

The classical rash in SLE is called the malar rash or butterfly rash. It is an erythematous, non-pruritic, non-scarring rash which extends over the nasal bridge, sparing the nasolabial folds. It can affect the chin and ears as well. The rash in SLE is frequently photosensitive, with flares of the rash heralding a flare of the systemic manifestations. Discoid rash is seen less frequently in pediatrics as compared to adults and it is a scarring erythematous scaly lesion which can mimic tinea lesions.

There are various other cutaneous manifestations of lupus including photosensitivity, Raynaud's phenomenon, alopecia, vasculitic rash, palmo-plantar/periungual erythema, and bullous lesions.(21)

In an inception cohort study of 256 SLE patients at the Hospital for Sick kids, Toronto, malar rash was seen in 66% of the patients, making it one of the most common clinical manifestations.(30) In their study arthritis was seen in 67%, nephritis in 55%, and CNS disease in 27% of the children.

Children can also present with oral or nasal hyperemia, shallow painless oral ulcers on the hard palate, or even nasal septal perforation.

Musculo-skeletal manifestations

Musculo-skeletal involvement in SLE can be due to active disease process or due to side effects of drugs used to treat the disease, including steroids. Children can present with arthralgia or arthritis, bone fragility, fractures and secondary pain amplification. Arthritis in children with SLE is one of the commonest presenting features. It is usually symmetrical polyarthritis, affecting both large and small joints. SLE can develop in children earlier diagnosed to have JIA.(25) Varying figures have been quoted for the incidence of arthritis in pediatric SLE. Levy et al found arthritis in 80% of children with SLE, while Hiraki quoted an incidence of 67%.(21,30) In a retrospective study published from Amrita Institute of medical

sciences,Kerala, arthralgia was found in 65% of children with SLE and it was one of the commonest presenting feature along with fever.(23)

Osteoporosis and avascular necrosis are frequently seen, and are attributed to corticosteroid use. However these manifestations are more commonly seen in children with SLE than with other similar illnesses which are treated with long term corticosteroids.

Hematological and immunological manifestations

Cytopenias are common in SLE and at least 50% of children present with decrease in atleast one cell line.(30) Leukopenia, along with lymphopenia and/or neutropenia can be a presenting feature and can be a marker of active disease or disease flares. Neutropenia can also be due to drugs used to treat the disease such as cyclophosphamide. Anemia is usually present and it can be – normocytic normochromic anemia of chronic disease, iron deficiency anemia or coombs positive hemolytic anemia due to autoantibodies. GLADEL study in Latin America showed hemolytic anemia in 15% patients.(31) Mild to severe thrombocytopenia is frequently seen, though it rarely requires transfusion. The risk of severe bleeding in SLE related thrombocytopenia is similar to the risk in immune thrombocytopenic purpura and hence transfusion is limited to patients with platelet count less than 20,000/cumm. Children with isolated thrombocytopenia, with a diagnosis of immune thrombocytopenic purpura should have screening tests done for SLE, as the initial manifestation may be limited to hematological abnormalities.(21)

Antiphospholipid antibodies are seen in almost 40% of children with SLE and are usually associated with hypercoagulability. However thrombotic or thromboembolic phenomenon are usually seen in less than half of these patients. Commonest manifestations are deep vein thrombosis, cerebral venous thrombosis and pulmonary thromboembolism.(32) Arterial thromboembolism is less frequent.

Neuropsychiatric manifestations

SLE can involve both central and peripheral nervous systems and there are various SLE related neuropsychiatric syndromes which are described. Up to 25% of children with SLE have neuropsychiatric manifestations and 70% of them present within the first year after diagnosis(33). Headache (66%), psychosis (36%), cerebrovascular disease (24%) and cognitive dysfunction (27%) are the commonest neurological manifestations. Children can also present with seizures, chorea or cerebrovascular accidents. Overall prognosis is generally good, with 95-97% survival, however about one fourth of children may have permanent sequelae.(33)

Lupus Nephritis

Renal disease can be seen in up to two thirds of children with SLE and presents mostly within the first two years after diagnosis(34). It is the main cause of morbidity and mortality in childhood SLE. Lupus nephritis in children is mostly asymptomatic initially and this underscores the need for careful systematic monitoring of urinalysis and blood pressure.

SLE mainly affects the glomerulus and presents as glomerulonephritis, however it can affect the renal interstitium as well. Manifestations of renal disease can range from minimal proteinuria and microscopic hematuria to frank renal failure and nephrotic syndrome. It can also present with features of thrombotic microangiopathy such as atypical hemolytic uremic syndrome or thrombotic thrombocytopenic purpura.(34)

As per the ACR criteria, lupus nephritis is defined as

- persistent proteinuria (> 0.5g/day , or spot UP/UC > 0.5 or >3+ by dipstick) and /or
- casts (cellular, RBC, granular or mixed casts)

Renal biopsy is the current gold standard for diagnosis of lupus nephritis. It is recommended when a child presents with any of the following:

- elevated creatinine without other explanation
- confirmed proteinuria
- Combinations of the following – proteinuria with hematuria or proteinuria with casts

In the study conducted by Hiraki et al in Toronto, lupus nephritis was seen in 55% of the study population. Children with renal and CNS disease had the highest SLE disease activity index (SLEDAI) scores (30). Kadar Ismail et al, reviewed the data for 39 children admitted with acute renal failure in four tertiary care hospitals in Somalia and found SLE to be the cause for ARF in 5% of cases

***Histology of Lupus nephritis*:**

The most recent classification for lupus nephritis is the one proposed by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) in 2003.They classified lupus nephritis into 6 histological types, based on light microscopy, electron microscopy and immunofluorescence. Of these class IV lupus nephritis – diffuse proliferative lupus, remains the most severe.(24, 25)

Class	Name	Clinical features	Pathological features
Class I	Minimal mesangial lupus nephritis	No renal findings	Normal light microscopy, minimal mesangial deposits in immunofluorescen
Class II	Mesangial proliferative L	Mild to moderate proteinuria	Hypercellularity and mild mesangial expansion on LM, mesangial deposits evident on immunofluorescence, subepithelial or subendothelial deposits on EM
Class III	Focal proliferative LN	Urinary sediments, greater proteinuria, hypertension	Lesions present in <50% glomeruli, classified as A – active lesions C – Chronic scarring lesions A/C – both active and scarred lesions
Class IV	Diffuse proliferative LN	Most severe renal involve active sediments, heavy proteinuria, often with reduced GFR. Serology mostly positive	Lesions in > 50% glomeruli, may be segmental or global, again classified as acute, chronic or acute/chronic
Class V	Membranous LN	Nephrotic range proteinuria with less active serology	Thickening of glomerular basement membrane, global or segmental immune deposits, may have mesangial expansion
Class VI	Advanced sclerosing LN	Renal failure	> 90% of glomeruli involved, no residual renal activity

Renal biopsy is the current gold standard for diagnosing lupus nephritis. However as this is an invasive procedure, it cannot be used for routine monitoring of renal status or progress of

the disease. Several clinical indices have been developed for adults with lupus nephritis, and these have been validated in children. Of these the SLEDAI – renal domain remains the best standard to monitor kidney function in children.

The SLEDAI-R score is the sum of 4 renal related items in the SLEDAI 2K score. Each item is given a score of 4, with a maximum total score of 16. 0 is considered as inactive lupus nephritis. Items in the SLEDAI-R score include – Cellular casts, proteinuria >0.5g/day, hematuria >5 RBC/hpf and pyuria >5 WBC/hpf

DIAGNOSIS OF SLE

The diagnosis of SLE requires detailed clinical and laboratory evaluation to establish the disease as well as to rule out other differentials such as malignancy. The most commonly used classification criteria for SLE was the American college of Rheumatology criteria (ACR criteria), which was initially published in 1982 and revised in 1997. According to this criteria, four of the following eleven criteria, simultaneously or cumulatively over time established the diagnosis of SLE, with sensitivity and specificity greater than 95% (23).

The ACR criteria for diagnosis of SLE are as follows:

1) Malar rash
2) Discoid rash
3) Oral or nasal ulcers
4) Photosensitivity
5) Arthritis - Non erosive, ≥2 joints
6) Serositis
7. Renal manifestations- Consistent renal biopsy - Persistent proteinuria or renal casts
8) Seizures or psychosis
9. Hematological manifestations Hemolytic anemia Leukopenia (Leukocytes < 4000/mm3) Lymphopenia (Lymphocytes< 1500/mm3) Thrombocytopenia (Platelets < 100,000/mm3)
10.Immunological criteria Anti ds-DNA or anti Smith positive False positive rapid plasma regain test, positive anticoagulant or elevated anti-cardiolipin
11. Positive antinuclear antibody test

***SLICC*:**Systemic lupus international collaborating clinics (SLICC) is an international group dedicated to SLE research, and they undertook a revision of the ACR SLE classification criteria. Data was reviewed from 716 patients from 25 sites and data for each patient was reviewed by at least 25 clinicians. A consensus diagnosis was achieved for 98% of the 716 patient data submitted for review and from this data, 17 criteria, which were associated with SLE diagnosis, were identified. These criteria were classified as clinical and immunological, and they are as follows:

Clinical criteria

1. Acute cutaneous lupus
    a. Including lupus malar rash
    b. Bullous lupus
    c. Toxic epidermal necrolysis variant of SLE
    d. Maculopapular lupus rash
    e. Photosensitive lupus rash – in the absence of dermatomyositis
2. Chronic cutaneous lupus
    a. Discoid rash – localized or generalized
    b. Hypertrophic (verrucous lupus)
    c. Lupus panniculitis
    d. Mucosal lupus
    e. Chilblains lupus
    f. Discoid lupus/lichen planus

3. Oral ulcers or nasal ulcers – in the absence of other causes such as vasculitis/Behcet/inflammatory bowel disease/reactive arthritis and acidic foods.
4. Non scarring alopecia – in the absence of other causes such as alopecia areata, iron deficiency and androgenic alopecia.
5. Synovitis involving two or more joints, or tenderness in two or more joints and thirty minutes of more of morning stiffness
6. Serositis for more than one day , in the absence of other causes such as infection, uremia and Dressler's pericarditis
7. Renal
    a. 24 hour urine protein more than 500 mg
    b. Red blood cell casts
8. Neurological
    a. Seizures
    b. Psychosis
    c. Mononeuritis multiplex in the absence of other known causes such as primary vasculitis
    d. Myelitis
    e. Peripheral or cranial neuropathy
    f. Acute confusional state in the absence of other causes including toxins, uremia or drugs
9. Hemolytic anemia
10. Leukopenia (< 4000/mm3) or lymphopenia (<1000/mm3) , atleast once

11. Thrombocytopenia (<100,000/mm3) atleast once

Immunological criteria

1. ANA above laboratory reference range
2. Anti- dsDNA above laboratory reference range
3. Anti-Smith
4. Antiphospholipid antibody: any of the following
    a. Lupus anticoagulant
    b. False positive RPR
    c. Medium or high titreAnticardiolipin (IgA, IgG or IgM)
    d. Anti β2 glycoprotein (IgA, IgG, IgM)
5. Low complement
6. Direct Coomb's test in the absence of hemolytic anemia

SLICC criteria for SLE require:

a) Four criteria, simultaneously or cumulatively, of which one is clinical and one immunological. Or
b) Lupus nephritis, with either ANA or anti-dsDNA positivity.

The new scoring system incorporates additional features such as low complements, Direct coombs test positivity and gives greater clarity for neurological and skin manifestations of SLE(37).

Experts found that use of SLICC classification resulted in fewer misclassifications than with ACR criteria. SLICC criteria also had better sensitivity (94% vs. 86%,

p<0.0001) and equal specificity (92% vs. 93%, p = 0.39) as compared to ACR criteria.(37)

MONITORING DISEASE ACTIVITY IN SLE – SLEDAI AND BILAG SCORES

Due to the protean manifestations of SLE, assessing patients at each visit with regard to disease remission or flare is complicated. There are various scoring systems that are used to measure the reversible inflammation in SLE, and these are internationally validated. Some of these scoring systems are British Isles Lupus Assessment group (BILAG), Systemic lupus activity measure (SLAM), European consensus lupus activity measure (ECLAM) and SLE disease activity index (SLEDAI). Of these the BILAG score is the most comprehensive and aims to show disease activity in individual organs. It is a transitional index, with each item measured as new, same, improving or worse.(38) However with 86 items, it is the longest of the SLE activity indices and requires time and training to perform.

SLEDAI score consists of 24 items, of which 16 are clinical questions related to various organs and systems. 8 of the 24 are laboratory results such as urinalysis, complement levels, ANA activity etc. SLEDAI is the shortest of the SLE disease scores and it does not contain subjective measures such as fatigue, joint pain etc. Scores are based on whether symptoms were present or absent in the past 10 days. Organ involvement is weighted, like neurological symptoms are multiplied by 8, while joint pain and kidney disease are multiplied by four. The weighted scores are summed into a final score, which can range from 0 – 105. A SLEDAI score more than 6 has been shown to be consistent with active disease requiring therapy. A clinically evident difference can be made out with an improvement of 6 points or

a worsening of 8 points.(39) There are three versions of SLEDAI – original version, SLEDAI 2k and MEX-SLEDAI. The MEX SLEDAI has the added advantage of avoiding costly investigations as it does not include complements and ds-DNA.

COMPLEMENT SYSTEM

The complement system consists of numerous plasma proteins , which interact with each other to opsonize pathogens and induce inflammatory responses which help the body to fight infections. It is the first line of defense against microorganisms and plays a very important role in both innate and acquired immunity. There are three pathways for initiation of the complement system, with a final common pathway. These three pathways are

a. Classical pathway activated by immune complexes
b. Alternative pathway activated by bacterial surfaces
c. Mannose binding lectin pathway

The common final pathway results in formation of a membrane attack complex. The first complement component C1, is composed of three subunits- C1q, C1r and C1s. C1qis a polypeptide multimer with 18 chains and a molecular weight of 460 kDa. C1q has to bind to atleast two heavy chains in order to change its conformation and activate the classical pathway.(6) Hence C1q is activated only by immune complexes containing immunoglobulins and multivalent antigens. Genes coding for the three arms of human C1q (A, B and C chains) are found on the short arm of chromosome 1 within the region

1p34.1 – 1p36.3. Mutations leading to homozygous C1q deficiency are inherited in an autosomal recessive manner and usually presents with a lupus like syndrome. (6)

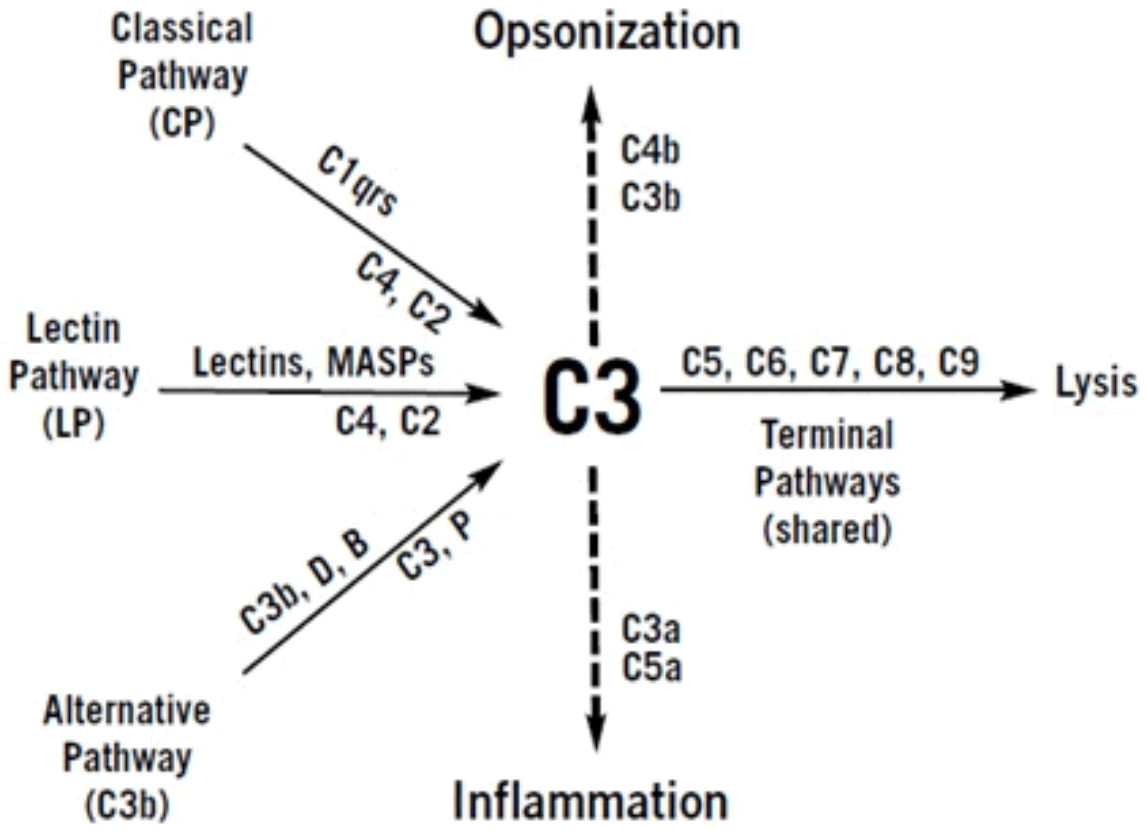
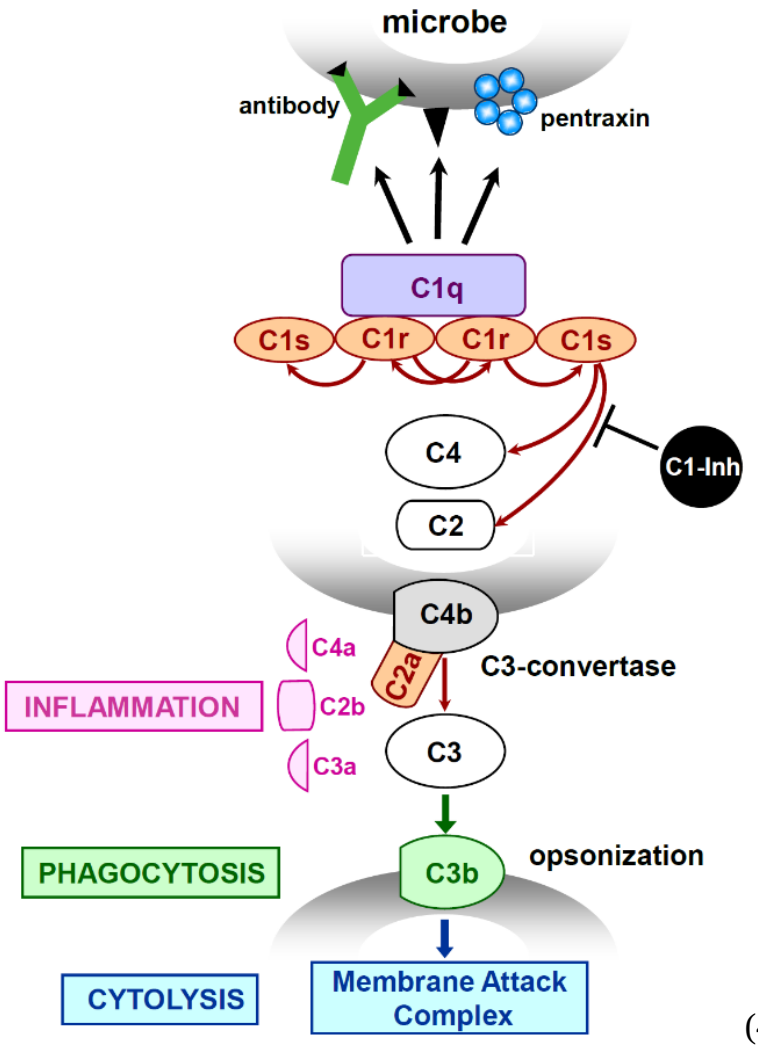


Fig 1: COMPLEMENT PATHWAYS



(40)

FIG 2: CLASSICAL COMPLEMENT PATHWAY LEADING TO CYTOLYSIS

C1q specifically binds to early apoptotic cells and clears dying cells by activating the classical complement pathway.

ANTI-C1q IN LUPUS NEPHRITIS

Complement pathway plays an important role in the pathogenesis of SLE, as proved by genetic predisposition to SLE in children with early complement pathway deficiencies. This also leads to the proposition that complement antibodies are associated with SLE. Most work in this field has been done on anti C1Q and its association with lupus nephritis.

Pathogenic association of anti C1q with SLE was demonstrated by the work of Mart Mannik and Mark Wener, which was first published in 1997. They obtained kidney biopsies at autopsy, of 12 patients who were diagnosed to have SLE. Antibodies to C1q collagen like region were found in 4 of the 12 patients. All four patients were found to have proliferative lupus nephritis. There was one patient with lupus nephritis, who did not have significant anti-C1q titers.(5) Anti-C1q antibodies are not specific for SLE and are found in numerous conditions. Other conditions with high anti-C1q titers include hypocomplementemic urticarial vasculitis (100%), scleroderma, rheumatoid arthritis(32%), undifferentiated connective tissue disease (94%) and Felty syndrome(76%) Patients with hepatitis C are also found to have high antibody titers.

Anti-C1q antibodies are present in nearly one third of patients with SLE, especially in those with renal involvement and high disease activity. In the presence of

high antibody titers, the levels of C1q, C3 and C4 are usually low. In patients with homozygous C1Q deficiency, 88% were found to have SLE and 30% developed glomerulonephritis.(2) Levels of C1q were found to be low in patients with SLE with glomerulonephritis flare. Nived et al assessed the outcomes in 52 Swedish adults with biopsy proven lupus nephritis and they found that treatment response at 6 months and levels of anti-C1q positively correlated with outcomes of disease.(41)

FQ Wu and colleagues investigated 90 pediatric SLE patients, 43 in active stage and 47 in remission. They demonstrated significantly higher levels of anti-C1q antibodies in children with active disease which had a positive correlation with SLE disease activity index (SLEDAI) score.(12)

Marto and Bertolaccini studied 151 patients with SLE, of which 77 patients had lupus nephritis, and their disease activity was categorized according to the BILAG (British Isles Lupus Assessment Group) renal score. They measured the sera of these patients for anti C1Q by enzyme immunoassay and found that patients with active lupus nephritis had a higher prevalence of anti-c1Q as compared to patients without lupus nephritis (74% vs. 32%, RR= 2.3, 95% CI 1.6 to 3.3, $p<0.0001$). They did not find significant difference between patients without lupus nephritis and those who had inactive nephritis. Levels of anti C1Q were also found to be higher in patients with nephritis as compared to patients without nephritis (36 U/ml vs. 7.3 U/ml, $p<0.001$). They further conducted a retrospective cohort study in 83 patients without renal disease, whereby anti C1Q was found to be positive in 33 patients (39%). Nine of these patients

went on to develop lupus nephritis. None of the patients who had a negative anti C1Q developed renal disease and hence the negative predictive value of the test was 100%. The authors found positive correlation of anti C1Q with anti dsDNA and a negative correlation with levels of C3 and C4, which are established markers of disease activity and flare. The authors hence concluded that anti-C1Q was positively associated with lupus nephritis and that monitoring anti C1Q could be useful in predicting renal flares of the disease.(7)

In an international, multicenter collaborative study, a sample of patients who were assembled to derive the Systemic Lupus collaborating criteria(SLICC), samples for anti- C1q testing were collected from 308 patients with SLE and 389 patients with other rheumatological illnesses. Anti- C1q was found in 28% of patients with SLE, and in 13% of controls with other rheumatological illnesses.(42)

Genetic and ethnic factors play a role in the levels of anti-C1q antibody titers. Asians are found to have higher titers of antibody to C1q as compared to Caucasians and Afro-Americans. Also younger people less than 35 years of age have higher antibody levels as compared to older individuals.

Kabeerdoss et al conducted a retrospective chart review of all SLE patients attending the rheumatology OPD of Christian Medical College, Vellore from March 2013 to January 2015. Clinical and laboratory data was retrieved from the electronic records of patients. Disease activity was scored according to SLEDAI scores and patients were classified as having mild, moderate or severe flare (SLEDAI score <8, 8-18

and >18 respectively). Renal SLEDAI score was used to assess the renal disease activity. Of the 126 patients included in the study, 54.76% of patients had lupus nephritis. 42.8% of SLE patients had positive anti-C1q titers, defined as anti c1q level >10U/ml, while 50.7% of the lupus nephritis subgroup had positive titers. The group found significantly higher levels of antibody in patients with lupus nephritis as compared to patients who had SLE without lupus nephritis. There was also a statistically significant difference between patients who had active lupus nephritis and those with quiescent disease. In multivariate analysis, mucocutaneous disease (OR 4.72), low C4 (OR 3.11) and higher renal SLEDAI scores (OR 1.35) were found to have significant correlation with positive anti-C1q antibody. If renal SLEDAI score was removed from the analysis, UP/UC ratio was found to attain significance (OR 1.77, $p<0.05$). This study further confirms the utility of C1q antibody as a novel biomarker for renal involvement in patients with SLE.(43)

Most studies evaluating C1q antibodies in SLE, and its correlation with lupus nephritis have been done in adults. Pediatric studies are few and far in between and there are no studies from India. Even though the etiopathogenesis of SLE in children and adults is similar, children differ from adults in their clinical manifestations and disease severity. Hence studies evaluating the role of this novel biomarker in Indian children with SLE are necessary.

METHODOLOGY

MATERIALS AND METHODS

Study population recruitment

This study was conducted in the Pediatric Rheumatology OPD and Pediatric wards from November 2016 to August 2017.

Inclusion criteria

All patients aged 6 – 18 years of age diagnosed with SLE according to the ACR criteria, attending the above clinic /ward, after obtaining parental consent

Exclusion criteria

a. Known case of congenital complement deficiencies
b. Other glomerulonephritis
c. Ambiguity in diagnosis

***Design of data collection*:**

This was a prospective cohort study. Data was collected from children fulfilling the inclusion criteria and entered in a proforma, which has been approved by the ethics committee of CMC, Vellore. Data was collected from hospital records and laboratory data which was available from the computerized hospital information system.

After obtaining parental consent, a portion of blood samples which were sent for various immunological tests was frozen and batches of blood samples were sent to the rheumatology laboratory.

Anti-C1q antibody assay was done by commercially available ELISA kit [IMTEC- anti-C1q-antibodies 9ITC590330, Germany]. Reference level of 20 IU/ml was taken as cut off as recommended by the manufacturers. A level of assay more than 20IU/ml was taken as positive.

Sample size calculation:

The required sample size to show that the prevalence of anti c1q with 95% confidence limits was found to be **267 SLE children** when the anticipated proportion of anti-C1q was taken as 30% based on previous studies with precision of about 5%.

The required sample size to show that anti-C1q was associated with renal involvement was found to be 223 SLE children with 80% power and 5% level of significance and anticipated odd's ratio of about 2 which was obtained from previous studies.

Statistical methods:

The categorical variables were analysed using frequencies and percentages. The prevalence of anti-C1q antibody was presented as percentage and 95% confidence interval. Continuous variables are presented as mean with standard deviation or median with interquartile range based on the distribution of data. The association of C1q with various clinical manifestations was obtained by calculating odd's ratio, adjusted for confounders by performing logistic regression analysis. The sensitivity and specificity with 95% confidence interval for anti-C1q antibodies in renal lupus was obtained. All statistical analysis were done using SPSS software version 17.0 or later. This study was approved by the Institutional Review Board dated 20/12/2016 (IRB Min No: 10305).

RESULTS

Table 1: Baseline characteristic features of children with SLE

Parameters		Number (%) n=150
Age	6 – 10 years	22 (15%)
	10 – 15 years	85 (57%)
Sex (M:F)		23 :127
Clinical features	Cutaneous	10 (6.7)
	Arthritis	13 (8.6)
	Serositis	1 (0.6)
	Seizures	6 (4)
	Psychosis	1 (0.6)
Lab parameters	Proteinuria	55(37)
	Leukopenia	19(13)
	Thrombocytopenia	6(4)
	Low C3	73 (49)
	Low C4	61 (41)
	DS DNA	79 (53)
Drugs used	HCQ	149 (97)
	NSAIDS	3 (2)
	Steroids	77 (51)
	MMF	53 (35)
	Cyclophosphamide	1 (0.6)
	Azathioprine	23 (15)
	Rituximab	7 (5)
Anti-C1Q positive		**95 (64)**
Renal biopsy		72 (48)

Table 2.Biopsies were done in a total of 72 children.Anti C1Q was positive in 59 (82%) of children who had undergone renal biopsy.

CLASS OF NEPHRITIS	NUMBER (%) (n=72)
CLASS II	7 (10)
CLASS III	21 (28)
CLASS IV	40 (54)
CLASS V	4 (6)

FIGURE 1

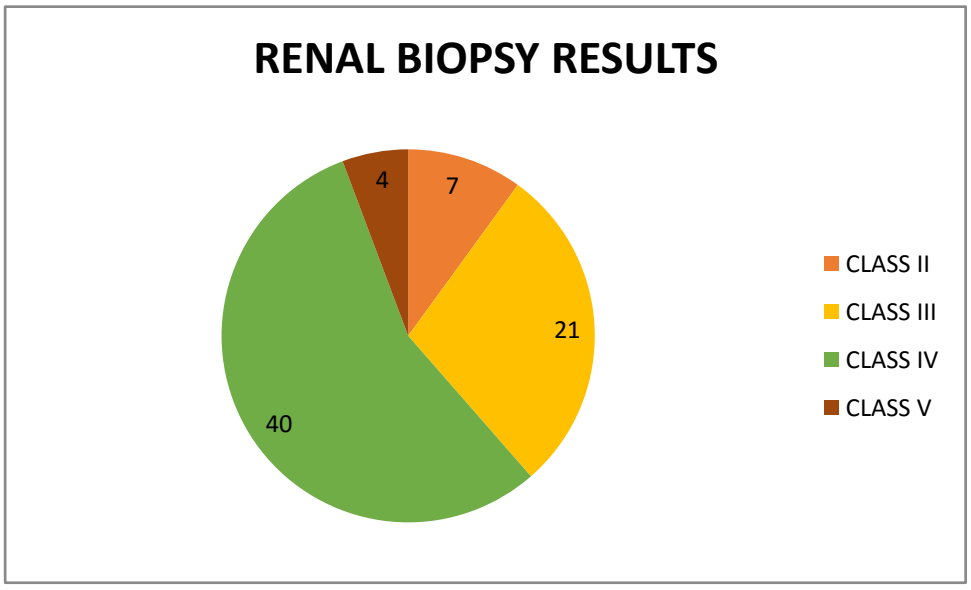


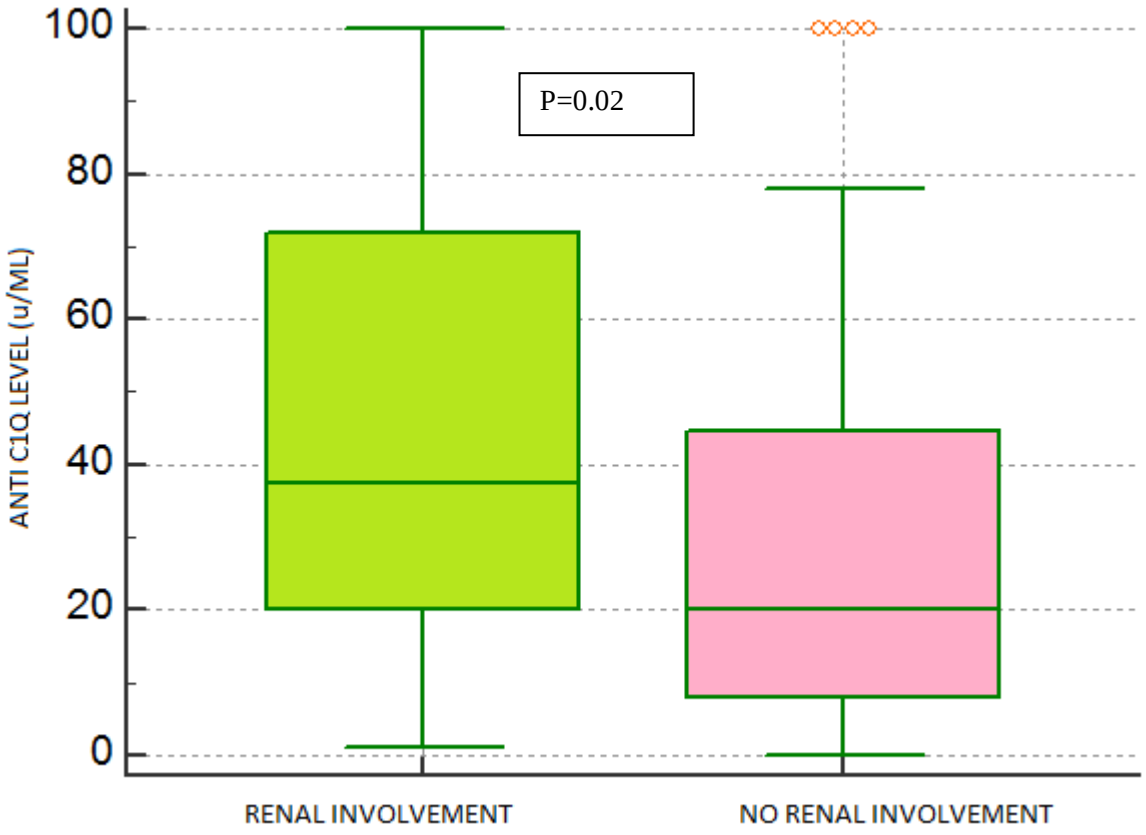
Table 3.Clinical and lab parameters compared with Anti-C1q status

Parameter	Anti–C1q positive N= 95	Anti-C1q Negative N=55	P value
Malar rash N(%) (n=10)	7 (70)	3 (30)	0.73
Arthritis N (%) (n=13)	11 (85)	2 (15.4)	0.13
Seizures N(%) (n=6)	2 (33)	4 (67)	0.2
PsychosisN(%) (n=1)	1 (100)	0 (0)	1
ProteinuriaN(%) (n=55)	42 (76)	13 (24)	0.009
LeukopeniaN(%) (n=19)	11 (58)	8 (42)	0.8
Thrombocytopenia N(%) (n=6)	3 (50)	3 (50)	0.67
Low C3N(%) (n=73)	58 (80)	15 (20)	<0.0001
Low C4N(%) (n=61)	49 (80)	12 (20)	<0.0001
Anti-dsDNAN(%) (n=79)	62 (78)	17 (22)	<0.0001

Table 4. Anti-C1q status in pSLE with or without renal involvement

	Renal involvement N (%) (n = 78)	No renal involvement N (%) (n = 72)	
Anti-C1q	58 (74%)	37(51%)	P=0.02

Figure 2: Anti-C1q status in pSLE with or without renal involvement



Anti- C1q antibody was positive in 58 (74%) in pSLE with renal involvement and was positive in 37(51%) in pSLE without renal involvement (p=0.02)

Table 5. Anti-C1q status in pSLE with active or inactive renal disease

	Active Renal disease (n =48)	Inactive renal disease (n = 30)	
Anti-C1q positive	42 (88%)	16(53%)	P=0.0015

Of the 78 children with lupus nephritis, 48 of them had active renal disease, defined as the presence of proteinuria, urinary cast or pyuria. Among the 48 children with active renal involvement, 42 had a positive value for anti-C1q (88 %), which was statistically significant.

Figure 3.Anti-C1q status in pSLE with active or inactive renal disease

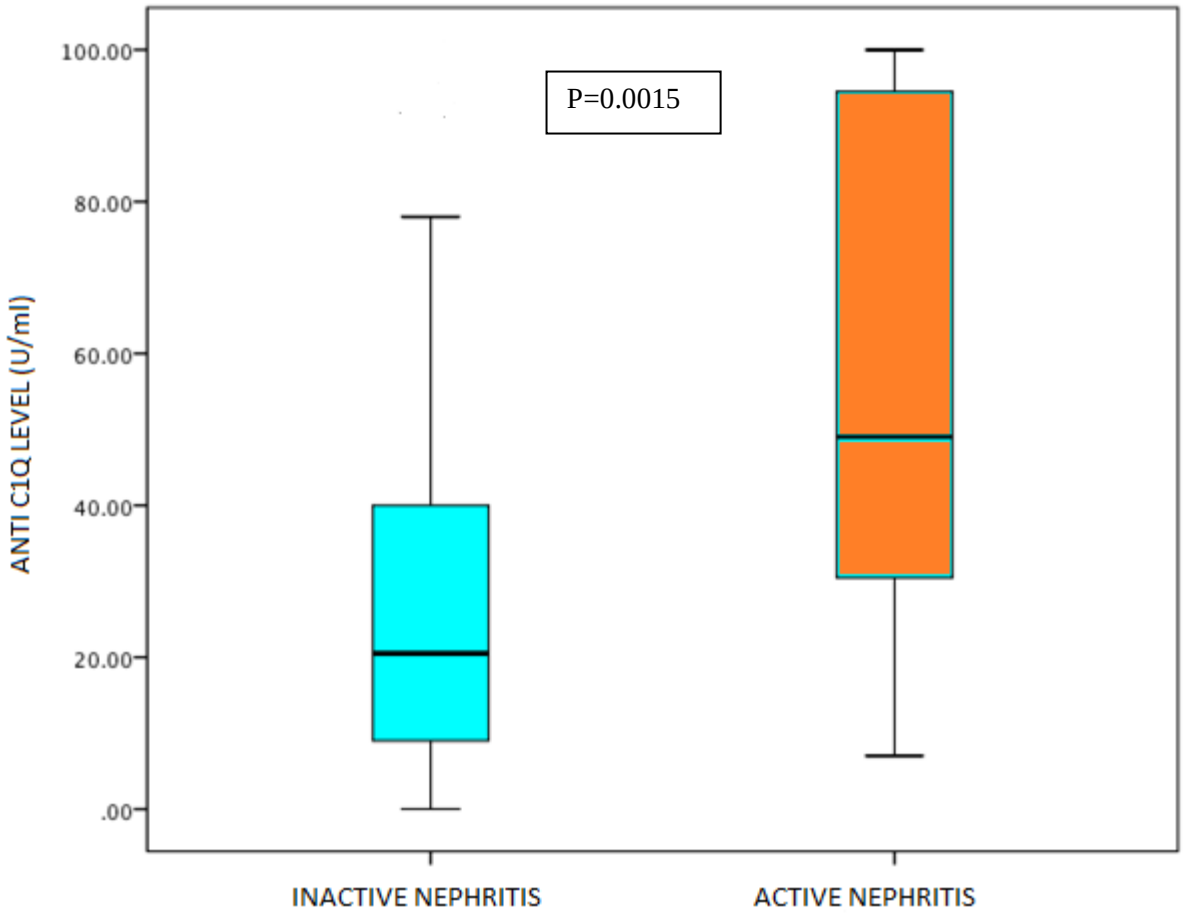


Table 6. Comparison of Anti-C1q in children who underwent renal biopsy

	Renal biopsy (n =72)	No renal biopsy (n = 78)	P value
Anti-C1q	54 (75 %)	39(50 %)	0.002

Anti-C1q was more likely to be positive in children who underwent renal biopsy than in those who did not, which was reflective of the association of anti C1q with renal disease

Table 7: Comparison of renal biopsy report with mean Anti-C1q level

Renal Biopsy	Number (n=72)	Mean Ani-C1q (U/ml)
Class II	7	15.29
Class III	21	49.85
Class IV	40	50.53
Class V	4	34

Class III and IV nephritis were commonest, and the mean Anti-C1q values were highest in these two groups.

Figure 4.Comparison of renal biopsy report with mean Anti-C1q level

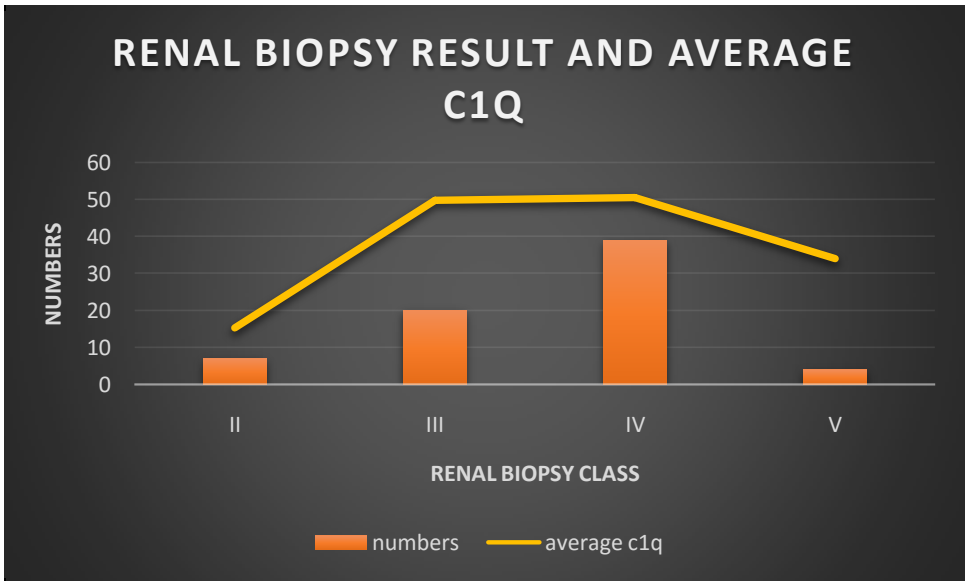
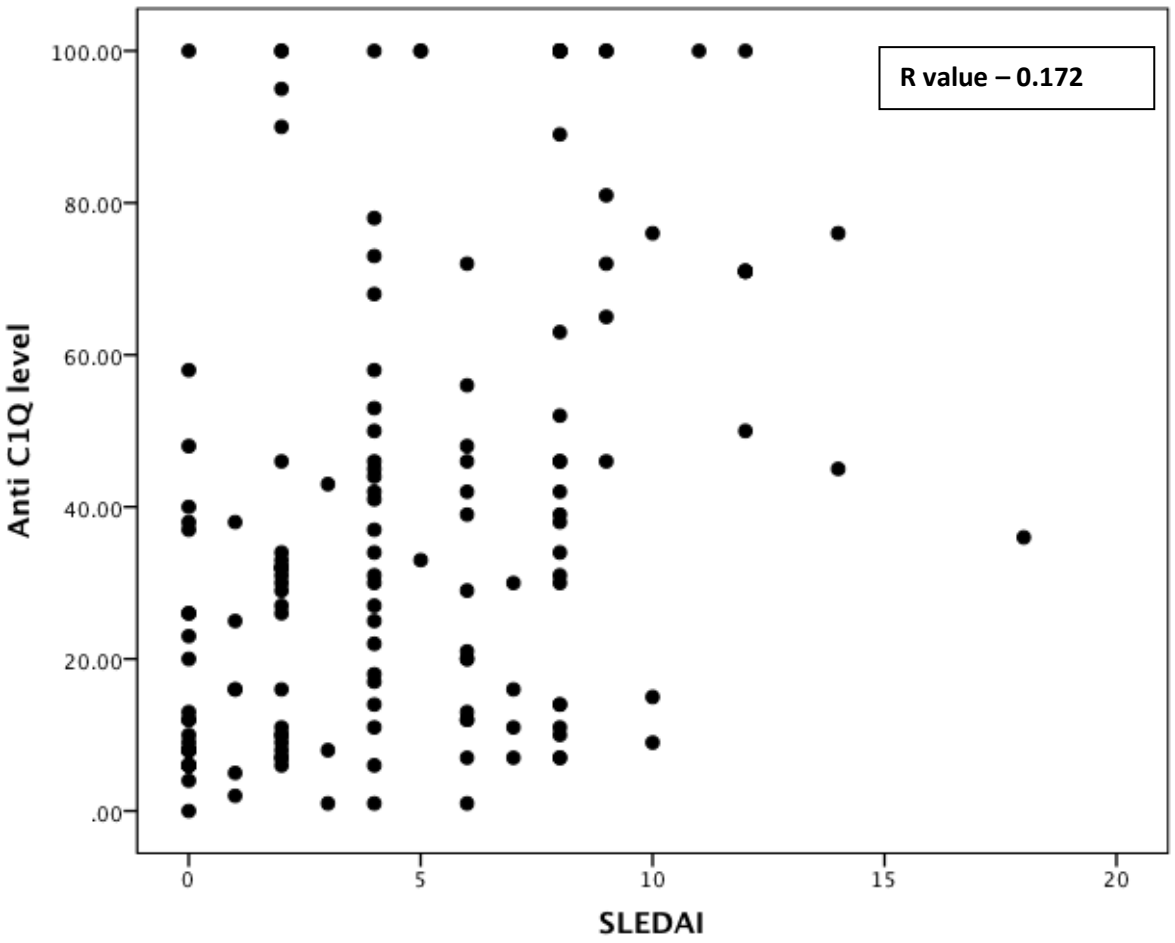


Figure 5.Correlation between positive SLEDAI and Anti-C1q antibody titers



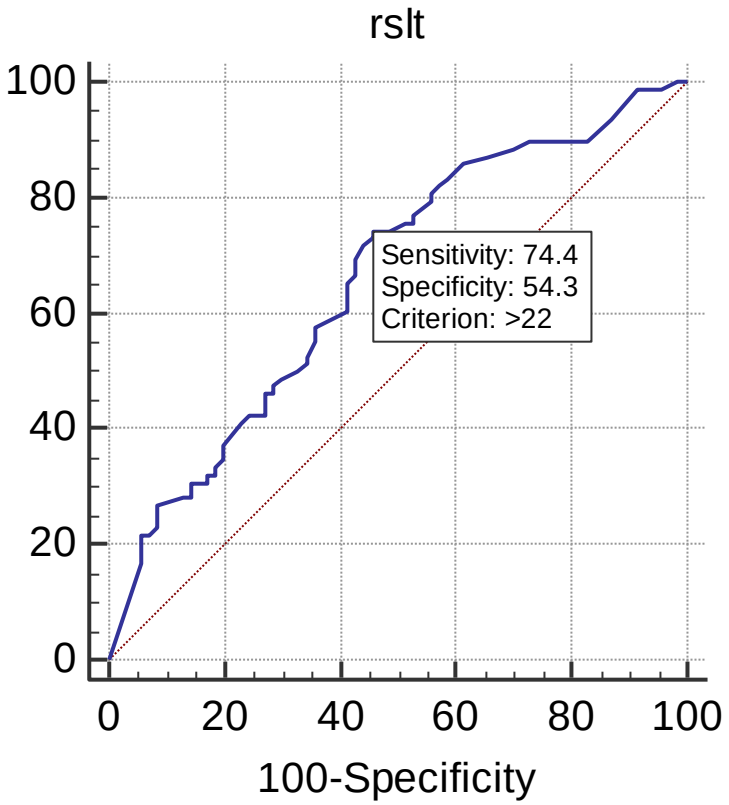
There was no significant correlation between average c1q antibody titers and SLEDAI positivity

Table 8.Correlation between positive SLEDAI and Anti-C1q antibody titers

	SLEDAI (>8) (n =43)	SLEDAI (<8) (n = 105)	P value
Mean Anti-C1q level (U/ml)	59	30	0.086

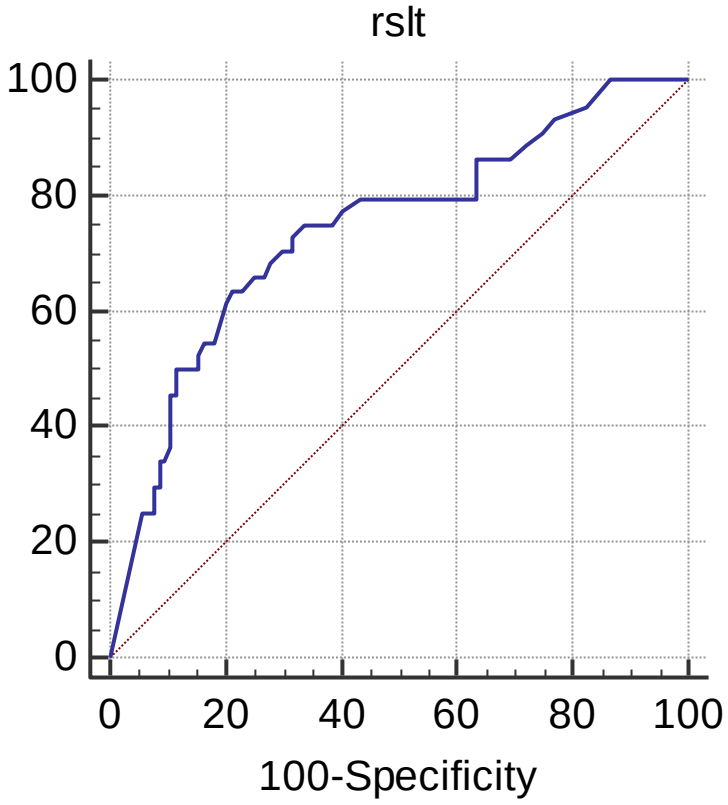
Mean Anti-C1q level was high with children with high SLEDAI (p=0.08)

Figure 6. ROC curve for sensitivity and specificity for Anti-C1q levels



Area under curve :0.659
Significance level : 0.0004
At anti C1q value > 22, sensitivity was 74.4% and specificity was 54.3%

Figure 7.ROC curve for sensitivity and specificity, taking SLEDAI as disease indicator



Area under curve = 0.742
Significance level = P<0.0001
At Anti-C1q value >44, sensitivity 63.64 and specificity 78.85

DISCUSSION

DISCUSSION

This observational study was performed to evaluate the diagnostic value of anti-C1q in the clinical follow-up of SLE patients with regard to disease activity in general and to renal involvement in particular. Between November 2016 to August 2017, 912 children attended the pediatric rheumatology clinic at Christian Medical College, Vellore. Of these, 271 Children had SLE and they constituted 30% of the total number of children. 150 children were recruited in the study and 121 children were excluded due to either lack of parental consent, age <6 years, or lack of adequate sample.

In our study, children in the age group of 11 to 15 years constituted more than half of the study population (57%), followed by children more than 16 years of age (29%). The mean age at onset for SLE in our study population was 11 years, which was similar to that seen in other studies, which reported a mean age at onset of 11 years (21,34). There was a significant female preponderance noted in the study with more than 80% of the study population being females. All the children with SLE less than 10 years of age were females. In the age group of 11-15 years, about 77% of the study population was females whereas in the age group more than 16, about 90% of the total number of patients were females.

Renal involvement was the most common clinical feature with 55 children having active proteinuria at the time of recruitment to the study and 78 children (52%) with established lupus nephritis. This is similar to reports published by Hiraki et al from Toronto, who

described a lupus nephritis incidence of 55% in their SLE population.(30) Sinha and Raut proposed that 50 – 75% of pediatric SLE patients have lupus nephritis.(34) They also found that lupus nephritis was more common in males than in females. However in our study, of the 78 patients with lupus nephritis, 68 were females and there were only 10 boys with lupus nephritis. The difference was not statistically significant.

Malar rash was seen in 10 children (6.67%) and no child had discoid rash. In the study from Toronto,(30) cutaneous manifestations were the most common, with malar rash being seen in 66% of the study population. In the study from West India, the incidence of malar rash was reported at 44%.(24)

Arthritis in our study was seen in 13 children (9%), which is again less than that reported previously from literature. Levy et al reported an arthritis incidence of 80% in pediatric SLE while Hiraki reported arthritis in about 67% of children.(21,30)Patwardhan et al also reported an arthritis incidence of 60% from West India, as did studies from the Amrita Institute in Kerala.(23,24) This low incidence of arthritis in our study may be attributed to the fact that majority of the children recruited were on follow up for many years and the disease was quiescent.

CNS manifestations are one of the most dreaded complications of pediatric SLE. In our study, there were 6 children with seizures and one child with psychosis. Hence CNS manifestations were present in 4.8% of the study population. In a study from South India on adult SLE, Robert and colleagues reported a seizure incidence of 20% and psychosis incidence of 16%.(44)

Low complement levels are commonly seen in SLE and are used as a marker of disease activity. Low complement levels are seen during disease flares. In our study low complements were seen in 77 children (51%). 79 children had anti dsDNA positive. 25 children had cytopenias(17%), which included mild to moderate thrombocytopenia and leukopenia. There were no children with severe anemia.

There were 78 (52%) children with lupus nephritis in our study population, of whom 48 children had active nephritis- defined as presence of a) urinary casts (heme-granular or RBC), b) proteinuria (>0.5g/24 hours, new onset proteinuria or increase >0.5g/24 hours) c) pyuria (>5 WBC/ hpf, excluding infection). Each criteria gets a score of 4 points.(45)

72 of the children with lupus nephritis underwent renal biopsy. Class IV lupus nephritis (Diffuse proliferative lupus nephritis) was the most common, followed by Class III (focal proliferative lupus nephritis). In the study from the Hospital for Sick Kids, Toronto, Class III and IV nephritis constituted about 80% of the renal biopsy samples.(30) The average anti-C1q values were found to be higher in Class III and IV lupus nephritis, reflecting a more severe disease course. There was however, no statistically significant difference between Class III and IV nephritis with regard to anti-C1q values.

Anti-C1q was found in the renal biopsy in 59 children with lupus nephritis. There was no significant difference between anti-C1q positivity among those with C1q in the renal biopsy and those without. There was also no statistically significant difference in the average C1q level between those with C1q in the renal biopsy and those without. Children with lupus nephritis were significantly more likely to have anti-C1q positivity

compared with children without renal involvement (74.4% vs. 48.6%, p=0.0001, OR 1.77, 95% CI 1.2 – 2.6). The mean anti-C1q value was also higher in the lupus nephritis group as compared to children without lupus nephritis (46.29U/L vs. 29.77U/L) and the difference was statistically significant (p=0.011). This is consistent with the study published by Marto and Bercolonni, where children with lupus nephritis were found to have higher anti-C1q values as compared to children without lupus nephritis.(7)

ANTI-C1q ANTIBODIES:

Antibodies to initial complement factors have been found to be increased in patients with SLE. Most of these studies have been undertaken in adults or mixed populations. In our study, 95 of 150 children (64 %) had positive C1q titers (> 20U/ml). In the study population assessed to formulate SLICC criteria, anti-C1q antibody positivity rate was 28%. In the study by Kabeerdoss et al (43)from our institution, 43% of SLE patients had positive C1q titers, taking a cut off of 10U/ml of ant-C1q as positive. At a cut off of 10U/ml, 120 children in our study had positive results (80%) of the study group. This percentage is much higher than that seen with adults.

Lupus nephritis was significantly associated with elevated anti-C1q antibodies (p=0.001, RR 1.7, 95%CI 1.2 – 2.6). 75.6% of lupus nephritis group had positive anti- C1q values. Published studies have found C1q positivity in 50 – 74%. Mean titers were also significantly increased in the lupus nephritis group as compared to those without renal involvement. This is again consistent with available literature. AUC analysis (area under curve) for positive anti-C1q titers for renal disease was 0.659, with sensitivity of 74% and specificity of 54% at anti-C1q cut-off of 22U/L. The positive and negative predictive value of anti-C1q titers in renal disease was both around 65%. In the retrospective study done by Picard et al, anti-C1q titers were positive in 84% of the children with active disease, and had better specificity than anti dsDNA in distinguishing patients with lupus nephritis (73% vs.19%).(46)

Proteinuria was present in 55 children, 44 of whom had positive antibody titers. Of the 95 children without proteinuria also, 51 had positive levels of anti-C1q antibody. However, the difference between the groups was statistically significant (p=0.0006, RR =1.5, 95%CI 1.19 to 1.87), though the average antibody titer was not significantly different between the groups.

In the study conducted by Kabeerdoss et al(43), cutaneous disease was also significantly associated with anti-C1q antibody titers. In our study we had 10 children with malar rash and there was no significant difference between those with cutaneous disease and without cutaneous disease with regard to antibody positivity. Average level of anti-C1q titers were also not significantly different between the two groups (42.59 vs. 38.14, p=0.89).

Monitoring disease activity in SLE clinically is done with the help of various scores, such as SLEDAI, BILAG and SLICC scores. Of these SLEDAI has been validated for use in children. SLEDAI score more than 8 is taken as positive for disease flare, with the following grading

1. SLEDAI <8 – mild disease
2. SLEDAI 8 -18 – moderate disease
3. SLEDAI >18 – severe flare

In our study 43 children had positive SLEDAI scores more than 8. There was no difference in anti-C1q positivity among those with or without positive SLEDAI (p=0.086). Comparing between the three groups, there were 105 children with SLEDAI

less than 8, 42 with SLEDAI score between 8 and 18, and one child with SLEDAI more than 8. Rates of C1q positivity were significantly different between the 3 groups with 60 positive in the mild group, 34 positive in the moderate group and 1 positive in the severe group (57.14%, 80.95% and 100% positive respectively). This difference was statistically significant (p=0.0187). However average C1Q antibody level was not statistically different between the three groups.

Low complement levels are used to assess disease flares in SLE. About half of our study population had low C3 or C4. There were 73 children with low C3 of who 58 had a positive anti-C1q value. C1q positivity was found to be significantly higher in children with low C3 as compared to normal C3 ($p<0.0001$, RR-1.8, 95% CI – 1.36 to 2.37). Mean C1q antibody level was also higher in the group with low C3 as compared to the group with normal C3 (53.9 vs. 23.5, $p= 0.001$). Similarly there were 61 children with low C4, of which 50 children had positive antibody titers, and the difference was statistically significant (p=0.0002, RR 1.6, 95%CI 1.25 to 2.01). However the average antibody titer between the groups was not significantly different (53.7 vs. 27.8, p=0.14).

Children with active lupus nephritis were more likely to have low C3 levels as compared to children with inactive lupus nephritis ($p =0.0028$, OR 2.25). Similarly the active lupus nephritis group had significantly higher number of children with low c4, as compared to inactive nephritis group (p=0.0012, OR 5.48). In a recently published clinical report from Peking University, Di Song and colleagues evaluated the role of complements in lupus nephritis. They found that plasma levels of C3 were lower in patients with active lupus

nephritis, than in those in remission (0.62±0.37 μg/ml vs. 1.09±0.18 μg/ml , p<0.001).(46) In a retrospective cohort study from Italy, Gandino and colleagues evaluated the role of complements in various clinical manifestations of SLE. They found that patients were persistently low complement levels were significantly more likely to have lupus nephritis than patients with normal complement levels(47).

Elevated anti-dsDNA levels are also associated with lupus flares. Of the 150 children in the study, anti-dsDNA was positive in 79 children (52.67%). Of these, 62 children had positive anti -C1q values. There was a statistically significant difference between children with anti-dsDNA positivity and those without (78.5% vs. 47.8%) (p=0.0004, RR 1.6 95% CI 1.25 to 2.15). Mean anti-C1q values were also different between children with elevated anti-dsDNA and those without. However the difference was not statistically significant (49.6 vs. 25.7, p=0.079). Anti-dsDNA is associated with disease pathogenicity and disease flares in lupus. In the study by Zivkovic et al from Serbia, active lupus nephritis was associated with higher anti-C1q positivity and anti-dsDNA co-positivity.(48) They found that anti-dsDNA positivity was higher in the group with active lupus nephritis, as compared to controls with inactive lupus nephritis and SLE without nephritis (88.9% vs. 78.26% vs. 62.3% respectively) and the difference was statistically significant.(48)

SUMMARY

SUMMARY

- In our study population, pSLE was more common among children aged 10 – 15 years than in younger children
- Girls were more affected than boys, with almost 6:1 female:male distribution
- Renal involvement was the commonest presenting feature, with 52% of the study population having lupus nephritis.
- Prevalence of Anti- C1q inpSLE was 64% (95/150), at a cut off value of 20U/ml
- Children with proteinuria, low C3, low C4 and anti dsDNA positivity had were significantly more likely to have anti-C1q antibody positivity.
- 74% of children with renal involvement had anti-C1qcompared to 51% of children without renal involvement (p= 0.02)
- Among the children with lupus nephritis, children with active renal disease had significant anti-C1q positivity than in children with quiescent disease (88% vs. 53% , p= 0.002)
- Mean anti-C1q antibody levels were also higher in children with lupus nephritis (Mean level – 46 U/ml) than in those without (Mean level – 30 U/ml), and among those with lupus nephritis, levels were higher in children with active renal disease (Mean level – 57 U/ml)
- Levels of anti-C1q antibodies were higher in children with SLEDAI score >8 (Mean level – 59 U/ml)

- Anti-C1q antibodies had a sensitivity of 74% and specificity of 54% at a cut off value of 22U/L , for renal disease

CONCLUSION

CONCLUSIONS

Our data support the usefulness of anti-C1q in SLE, especially in lupus nephritis. Our study results revealed that Anti-C1q antibody titers were found to have positive correlation with renal disease in pSLE. Anti-C1q can be used as follow-up marker in SLE patients, in particular in SLE patients with renal involvement.Hence uses of anti-C1q determinations become important for clinical care and disease prognosis, anti-C1q can be reconsidered for inclusion in classification criteria and in the clinical management of SLE. Further studies has to be performed using well-defined large cohorts of patients with close as well as long follow-ups in order to determine the diagnostic value of anti-C1q in SLE patients.

LIMITATIONS

LIMITATIONS

Our study bears some important limitations.

Retrospective character of our analysis and the relatively small number of patients, in particular when comparing subgroups of patients, do not allow drawing definite conclusions.

Our study characteristics did not sufficiently allow the calculation of the sensitivity and specificity of anti-C1q for the determination of a specific nature and severity of flares in comparison to other laboratory markers of disease.

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ANNEXURES

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

December 20, 2016

Dr. George Ipe Vettiyil,
PG Registrar,
Department of Child Health II,
Christian Medical College,
Vellore - 632 004.

Sub: **Fluid Research Grant NEW PROPOSAL:**
Prevalence and clinical associations of Anti C1Q antibodies in children with SLE
Dr. George Ipe Vettiyil , Employment Number: 32610, Child Health II, PG Registrar, Dr. Sathish Kumar , Employment Number: 20174, Child Health II, Dr. Jayakanthan, Employment number – 31730 Rheumatology, Mrs. Visalakshi Jeyaseelan, Employment number – 31093, Biostatistics

Ref: IRB Min No: 10305 [DIAGNO] dated 12.10.2016

Dear Dr. George Ipe Vettiyil,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Cc: Dr. Sathish Kumar, Dept. of Child Health - II, CMC, Vellore

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
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Chairperson, Ethics Committee.

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Ref: IRB Min No: 10305 [DIAGNO] dated 12.10.2016

Dear Dr. George Ipe Vettiyil,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Prevalence and clinical associations of Anti C1Q antibodies in children with SLE" on October 12th 2016.

The Committee reviewed the following documents:

1. IRB Application format
2. Consent forms and Information Sheet (Tamil, English, Hindi),
3. Cv's of Drs. Visalakshi, George, Jayakanthan and Sathish.
4. No. of documents 1 – 3.

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on October 12th 2016 in the BRTC Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Name	Qualification	Designation	Affiliation
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, Research), Additional Vice Principal , Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC,Vellore	Internal, Clinician
Dr. B. J. Prashantham	MA(Counseling Psychology), MA (Theology), Dr. Min (Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Dr. Ratna Prabha	MBBS, MD (Pharma)	Associate Professor, Clinical Pharmacology, CMC, Vellore	Internal, Pharmacologist
Dr. Rekha Pai	BSc, MSc, PhD	Associate Professor, Pathology, CMC, Vellore	Internal,Basic Medical Scientist
Rev. Joseph Devaraj	BSc, BD	Chaplaincy Department, CMC, Vellore	Internal, Social Scientist
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Dr. Santhanam Sridhar	MBBS, DCH, DNB	Professor, Neonatology, CMC, Vellore	Internal, Clinician
Mrs. Sheela Durai	MSc Nursing	Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse
Ms. Grace Rebekha	M.Sc., (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Mrs. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay Person
Dr. Vivek Mathew	MD (Gen. Med.) DM (Neuro) Dip. NB (Neuro)	Professor, Neurology, CMC, Vellore	Internal, Clinician
Dr. Balamugesh	MBBS, MD(Int Med), DM, FCCP (USA)	Professor, Pulmonary Medicine, CMC, Vellore	Internal, Clinician

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
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Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
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Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Dr. Sneha Varkki	MBBS, DCH, DNB	Professor, Paediatrics, CMC, Vellore	Internal, Clinician
Mrs. Emily Daniel	MSc Nursing	Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse
Dr. Sathish Kumar	MBBS, MD, DCH	Professor, Child Health, CMC, Vellore	Internal, Clinician
Dr. Inian Samarasam	MS, FRCS, FRACS	Professor, Surgery, CMC, Vellore	Internal, Clinician
Dr. Thomas V Paul	MBBS, MD, DNB, PhD	Professor, Endocrinology, CMC, Vellore	Internal, Clinician

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: "Prevalence and clinical associations of Anti C1Q antibodies in children with SLE" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

Fluid Grant Allocation:

A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an Ist Installment. The rest of the 50,000/- INR (Rupees Fifty Thousand only) each will be released at the end of the first year as 2 nd Installment.

Yours sincerely,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

CHRISTIAN MEDICAL COLLEGE, VELLORE
AGREEMENT TO BE SIGNED BEFORE RELEASE OF ANY RESEARCH GRANT
*****

1. I understand that the research grant is sanctioned only for the specific project approved by the Institutional Review Board and should be used exclusively for this project

2. I note that the project will become operational with effect from the date on which the grant is received, and I agree to complete it within the stipulated time of 24 months.

3. I agree to submit promptly and regularly, the periodical (Half Yearly for One Year Project/Annually for Two years project) reports and the final report of the work done, in the approved format.

4. If I plan to leave the institution on before the completion of the project. I will submit a complete and detailed report of the work done by me on the project till the date of relief and transfer the project, either to the Guide or to the Co-Investigator for completion and submission of the Final Report.

5. I agree that any publication arising out of this project will carry an acknowledgement of the financial support of the Christian Medical College Fluid Research Fund.

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A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an Ist Installment. The rest of the 50,000/- INR (Rupees Fifty Thousand only) each will be released at the end of the first year as 2 nd Installment.

PRINCIPAL

Dr. BIJU GEORGE
MBBS, MD, DM,
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Dr. George Ipe Vettiyil,
Child Health - II.

Dr. Sathish Kumar, 21/2017
Child Health - II.

Project Title: Prevalence and clinical associations of Anti C1Q antibodies in children with SLE.

Ref: IRB Min No: 10305 [DIAGNO] dated 12.10.2016.

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PRINCIPAL

Dr. BIJU GEORGE
MBBS., MD., DM.,
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Dr. George Ipe Vettiyil,
Child Health - II.

Dr. Sathish Kumar, 21/2017
Child Health - II.

Project Title: Prevalence and clinical associations of Anti C1Q antibodies in children with SLE.

Ref: IRB Min No: 10305 [DIAGNO] dated 12.10.2016.

2. PROFORMA

ANTI C1Q ANTIBODIES IN SLE- PROFORMA

1. Serial number
2. Name
3. Hospital number
4. Sex
5. Address
6. Duration of symptoms
7. Date of first visit
8. Organs involved:

	Yes	No
Skin		
Renal		
CNS		
Hematological		
Musculoskeletal		
GI		

9. Renal involvement : Yes/No
10. Renal biopsy: Yes/No

11. Date of renal biopsy
12. Renal biopsy report
13. Stage of lupus nephritis

14. Date of current visit:
15. Current medication:

HCQ	
NSAIDS	
Steroids	
MMF	
Cyclophosphamide	
Biological	

16. Initial C3/C4
17. Current C3/C4
18. Antibodies positive
19. Anti C1q level:

3. CONSENT FORMS

CONSENT TO TAKE PART IN A CLINICAL TRIAL

Study Title: Study on diagnostic accuracy of Anti C1Q in children with SLE

Study Number:

Participant's name:

Date of Birth / Age (in years):

I_____

_____, father of _____

(Please tick boxes)

Declare that I have read the information sheet provide to me regarding this study and have clarified any doubts that I had. [ ]

I also understand that my child's participation in this study is entirely voluntary and that I am free to withdraw my child to participate at any time without affecting my usual treatment or my legal rights [ ]

I also understand that during the period of the study, Anti C1Q blood test is done free. But after this, if blood tests are prescribed, I may have to pay for it [ ]

I understand that my child will receive free treatment for any study related injury or adverse event but I will not receive and other financial compensation [ ]

I understand that the study staff and institutional ethics committee members will not need my permission to look at my child's health records even if I withdraw from the trial. I agree to this access [ ]

I understand that my child's identity will not be revealed in any information released to third parties or published [ ]

I voluntarily agree to take part in this study [ ]

Name: Principal Investigator:

Signature:

Date:

Signature:

Date:

Name of witness:

Relation to participant:

Date:

सहमति प्रपत्र

SLE में CLQ प्रतिरक्षी के प्रभाव पर अध्ययन

क्रमांक :-

प्रतिभागी का नाम :-

जन्म तिथी / उम्र :-

मैं _____, _____ की बेटी / का बेटा, यह मानता हूँ कि

(1) SLE में CLQ प्रतिरक्षी के प्रभाव पर दी गई सूचना पत्रिका मैंने पढी है और इस अध्ययन के बारे में जो संदेह थे, वह स्पष्ट किए हैं।

(2) इस अध्ययन में मेरी सहभागिता पूर्ण रूप से स्वैच्छिक है और मैं अपनी भागीदारी किसी भी समय वापस ले सकता हूँ। इससे मेरे इलाज़ पर कोई असर नहीं पडेगा।

(3) इस अध्ययन के दौरान CLQ प्रतिरक्षी का अनुमान भुगतान के बेगैर किया जाएगा, मगर इस अध्ययन के बाद अगर यह परीक्षण करना है, तो इसका मूल्य चुकाना होगा।

(4) इस अध्ययन के कारण अगर मुझे कोई नुक्सान होता है, तो उसका कीमत मुझे दिया जाएगा मगर उसके अलावा और कुछ पैसे नही दिए जाएँगे।

(5) CMC कर्मचारी मेरा चार्ट देख सकते हैं और अगर मैं इस अध्ययन से भागीदारी वापस लेता हूँ, तब भी वह मेरा चार्ट देख पाएँगे। मैं इससे सहमत हूँ।

(6) मेरा व्यक्तिगत पहचान किसी दूसरे से बाँटा नहीं जाएगा और ना ही प्रकाशित किया जाएगा।

मैं इस अध्ययन में भाग लेने के लिए स्वैच्छा से सहमति देता हूँ / देती हूँ।

नाम :

हस्ताक्षर :

तारीख :

सहमति पत्र

SLE में CLQ प्रतिरक्षी के प्रभाव पर अध्ययन

क्रमांक :-

प्रतिभागी का नाम :-

जन्म तिथी / उम्र :-

मैं, _____ का पिता / अभिभावक यह मानता हूँ कि

(1) मैंने SLE में CLQ प्रतिरक्षी के अनुमान के बारे में दी गई सूचना पत्रिका पढी है और इस अध्ययन के बारे में मुझे जो संदेह थे, वह स्पष्ट हो गए हैं।

(2) मैं यह जानता हूँ कि इस अध्ययन में मेरे बच्चे की सहभागिता पूर्ण रूप से स्वैच्छिक है और मैं अपनी भागीदारी किसी भी समय वापस ले सकता हूँ। इससे मेरे बच्चे के इलाज पर कोई प्रभाव नहीं पडेगा।

(3) मैं समझता हूँ कि इस अध्ययन के दौरान CLQ प्रतिरक्षी का अनुमान भुगतान के बगैर किया जाएगा, मगर इस अध्ययन के बाद अगर यह परीक्षण करना है, तो इसका मूल्य चुकाना होगा।

(4) इस अध्ययन के कारण अगर मुझे या मेरे बच्चे को कोई नुकसान होता है, तो उसकी कीमत मुझे दी जाएगी, मगर इसके अलावा और कुछ वैसे नहीं दिए जाएँगे।

(5) मैं यह समझता हूँ कि CMC कर्मचारी मेरा चार्ट देख पाएँगे और अगर मैं इस अध्ययन से भागीदारी वापस लेता हूँ, तब भी वह मेरा चार्ट देख पाएँगे। मैं इसके लिए अनुमति देता हूँ।

(6) मैं समझता हूँ कि मेरे बच्चे की व्यक्तिगत जानकारी या पहचान किसी भी रूप में प्रकाशित नहीं की जाएगी और किसी और से बाँटा नही जाएगा।

मैं इस अध्ययन में भाग लेने के लिए स्वेच्छा से सहमति देता हूँ।

4. MASTER COPY

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#age           1   5  30  26   5   0   2 112 age     age of patient
_hno           1   7  30  27   7   1   7 112 hno     hospital number
#sex           1   9  30  29   9   0   1 112 sex     gender of patient
_label1       30   9  30   0   0   0   0 112     1-male 2-female
#adrs1  11  30  28  11   0   1 112 adrs    address
_label2       29  11  30   0   0   0   0 112     1-Tamil Nadu, 2-Andhra Pradesh, 3-West Bengal, 4-Jharkhand, 5-Bihar, 6-Keral
_label3      109  11  30   0   0   0   0 112 a, 7-Karnataka, 8-Bangladesh, 9-Others
_add           1  13  30  30  13   1  25 112 add    address of patient
#dur1  15  30  32  15 102   5 112 dur    duration of symptoms
_label4       37  15  30   0   0   0   0 112     yy.mm
_dtdg1  17  30  30  17  11  10 112 dtdg    date of diagnosis
_dtvst1  19  30  32  19  11  10 112 dtvst    date of last visit
#fu1  21  30  32  21 102   5 112 fu    duration of follow up
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_dsrs1  26  30  25  26   5   1 112 dsrs    discoid rash
_oa1  28  30  22  28   5   1 112 oa    oral ulcers
_arth1  30  30  22  30   5   1 112 artharthtitis
_srst1  32  30  22  32   5   1 112 srstserositis
_plrs1  34  30  21  34   5   1 112 plrs    pleurisy
_prcd1  36  30  25  36   5   1 112 prcd    pericarditis
_prtu1  38  30  24  38   5   1 112 prtu    proteinuria
_rbcc1  40  30  27  40   5   1 112 rbcc    red cell casts
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_psc1  44  30  21  44   5   1 112 psc    psychosis
_hmt1  46  30  23  46   5   1 112 hmt    hematologic
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_lmpp1  50  30  24  50   5   1 112 lmpp    lymphopenia
_tbcp1  52  30  29  52   5   1 112 tbcp    thrombocytopenia
_lc3           1  54  30  18  54   5   1 112 lc3    low c3
_lc4           1  56  30  18  56   5   1 112 lc4    low c4
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_ansm1  60  30  22  60   5   1 112 ansm    ant smith
_lpac1  62  30  29  62   5   1 112 lpac lupus anticoagulant
_aclp1  64  30  29  64   5   1 112 aclp    anti cardiolipin
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#slc1  68  30  17  68   0   2 112 slc    SLICC
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_active        1  72  30  39  72   5   1 112 active    Active renal involvement
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#rbxr1  78  30  32  78   0   1 112 rbxr    renal biopsy result
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_c1qbx         1  80  30  38  80   5   1 112 c1qbx    Anti C1Q in renal biopsy
_hcq1  83  30  15  83   5   1 112 hcq    HCQ
_nsd1  85  30  17  85   5   1 112 nsd    NSAID
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_mmf1  89  30  15  89   5   1 112 mmf    MMF
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_rtx1  95  30  21  95   5   1 112 rtx    rituximab
#rslt1  98  30  27  98 102   5 112 rslt    Anti C1Q level
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61KEERTHIKA 10556189G21VELLORE, TN 0.0201/04/201701/0!
6/2017 0.02NNNNNNNNNNNNNNNNYYYNNN 4 NNN YNYNNNN78.00!
62GUNASUNDARI 15013294G21KANCHEEPURAM, TN 3.0001/07/201401/0!
6/2017 3.00NNNNNNNNNNNNNNNNNNNNN 0 NNN YYNNNNN 9.00!
63AMRITHA 8713280G21KARUR, TN 1.0001/10/201601/0!
6/2017 1.00NNNNNNNYNNNNNNNNNYNNN 6 NNN YNYNNNN39.00!
64AYSHA 18409300F21VELLORE, TN 5.0001/06/201201/0!
4/2017 4.00NNNNNNNNNNNNNNNNNYNNN 2 YNY22/02/20133YYNNNNYN 9.00!
65SRIKARI 13327425G22VIZAG, AP 4.0001/12/201301/0!
6/2017 2.00NNNNNNNNNYNNNNNNNNNNN 8 YNN YNNYNNY10.00!
66RUHSA MARY 14313186F21TUTICORIN, TN 5.0001/05/201201/0!
3/2017 5.00NNNNNNNNNNNNNNNNNNNN 2 NNN YNNYNNN11.00!
67G PRASANTH 11617124F11VELLORE, TN 1.0001/06/201601/0!
7/2017 1.00NNNNNNNYNNNNNNNNYYYNNN 8 YYY18/07/20174YYNYYNNN39.00!
67PRATIKA 14765795F23WB 3.0001/01/201401/0!
3/2017 3.00NNNNNNNYNNNNNNNNYYYNNN 8 YYY31/12/20134YYNNYNNN 7.00!
69SUDIKSHA BARNAWAL 10771763G23WB 1.0001/08/201601/0!
3/2017 0.08NNNNNNNYNNNNNNNNNNNNNN 4 YYY20/01/20175YYNYYNNN37.00!
70BABY QUEENA 12551378G26TRIVANDRUM, KERALA 0.0601/03/201701/0!
4/2017 0.06NNNNNNNNNNNNNNNNNNNN 2 YNY24/03/20174YYNYYNNN34.00!
71MEENA 12300894G21TIRUVALLUR, TN 3.0001/01/201401/0!
6/2017 3.00NNNNNNNYNNNNNNNNYYYNNN 8 YYY04/09/20154YYNYYNNN99.99!
72AMILI UMPO 12397637G29ARUNACHAL PRADESH 1.0601/01/201631/0!
3/2017 1.06NNNNNNNYNNNNNNNNYYYNNN 8 YYY30/12/20163YYNYYNNN31.00!
73RAJESH 18054622F11VELLORE, TN 6.0001/10/201101/0!
6/2017 6.00NNNNNNNNNYNNNNNNNNNNN 8 NNN YNNNNNN14.00!
74SHASRIKA 5612725G21KARUR, TN 1.0001/06/201601/0!
3/2017 1.00NNNNNNNNNNNNNYNNNNNNN 1 NNN YNNNNNN 5.00!
75SHISTRI RAI 13430261G29SIKKIM 1.0601/11/201501/0!
4/2017 NNNNNNNNNNNNNYNNNNNNNN 1 NNN YNNNNNN16.00!
76ANTARA CHAKRABORTY 18010691F23WB 5.0001/05/201201/0!
4/2017 5.00NNNNNNNNNNNNNNNNNNNNN 0 NNN YNNNNNN12.00!

77TANIA DAS 18901219D23WB 6.0001/11/201101/0!
5/2017 6.00NNNYNNYNNNNNNNNNYYYNNN 10 NNN YNNNNNN 9.00!
78MARIYA ANGELIN 10841625G21COIMBATORE, TN 0.0601/01/201701/0!
4/2017 0.06NNNNNNNYNNNNNNNNNNNNN 4 NNN YNYNNNN31.00!
79AFRUUJAA AKTHER 13757907F28BANGLADESH 3.0001/06/201401/0!
5/2017 3.00NNNNNNNYNNNNNNNNYYNNNN 6 NNN YNNNNYN20.00!
80AISHWARYA 15071104G21COIMBATORE, TN 3.0001/10/201401/0!
5/2017 3.00NNNNNNNNNNNNNNNNYYYNNN 4 YYY20/10/20144YYNYYNNN42.00!
81SARUBALA 11338588F21KARUR, TN 5.0001/08/201201/0!
5/2017 5.00NNNNNNNNNNNNNNNNYYYNNN 4 NNN YNNNNNY50.00!
82SASI PEDAPUDI 12214146G22AP 2.0001/02/201501/0!
6/2017 1.00NNNNNNNNNNNNNNNNNNNNN 0 NNN YNNNNNN13.00!
83SABRITI DAS 13876325G23EAST MIDNAPORE, WB 1.0001/01/201701/0!
7/2017 0.06NNNNNNNYNNNNNNNNYNNNNN 6 NNN YNYNNNN20.00!
84SUVECHA PAL 16855873G23HOOGLY, WB 0.0601/02/201701/0!
6/2017 0.06YNNYNNNYNNNNNNNNYYYNNN 14 YYY24/04/20173YYNYYNNN76.00!
85KH PRIYA 17034688G22GUNTUR, AP 3.0001/01/201401/0!
5/2017 2.00NNNNNNNYNNNNYNNNNNNNN 5 YYY18/08/20153YYNNYNNY99.99!
86LAMTA 858602G 26MALAPPURAM, KERALA 1.0001/12/201601/0!
7/2017 1.00NNNNNNNNNNNNNNNNYNYNNN 4 NNN YNYNNNN53.00!
87JAYASHRI 15722501G21NAMAKKAL, TN 1.0001/12/201601/0!
6/2017 1.00NNNNNNNYNNNNNNNNYYYNNN 8 YYY28/07/20174NYNYNNYN30.00!
88DABITHA 14972307F22CHITTOOR, AP 2.0001/12/201501/0!
6/2017 2.00NNNNNNNNNNYYNNNNNNYNNN 18 YNN YNNNYYN36.00!
89VISHNU 13991498F12CHITTOOR,AP 1.0001/03/201601/0!
4/2017 1.00NNNNNNNNNNNNNNNNYYYNNN 4 NNN YNNNNNN45.00!
90DIVYA 17240422F21CHENNAI, TN 5.0001/09/201201/0!
5/2017 5.00NNNNNNNNNNNNNNNNNNNNN 0 YNN YNNNNNN12.00!
91DEBOLINA 10445453G23WB 1.0001/04/201610/0!
7/2017 1.00NNNNNNNNNNNNNNNNNNNNN 0 YNY28/03/20164YYNNYNNN20.00!
92RAGINI 17214711G29CHATTISGARH 2.0001/03/201501/0!
6/2017 2.00NNNNNNNNNNYNNNNNNNNNN 8 NNN YNNNNNN11.00!
93DEENA 13415254G26MALAPPURAM, KERALA 1.0001/06/201601/0!
5/2017 1.00NNNNNNNNYNNNNNNNNYYYNNN 8 YYY 3YYNYYNNN99.99!
94SARU 12338588F21KARUR, TN 2.0001/05/201501/0!
7/2017 2.00NNNNNNNNYNNNNNNNNYYYNNN 8 NNN YNNNNNY99.99!
95VISHNOO 12190733G12KADAPA, AP 2.0001/04/201501/0!
6/2017 2.00NNNYNNNYNNNNNNNNYYYNNN 12 NNN YYYNNNN71.00!
96SWETA 16742880F22KADAPA, AP 4.0001/06/201301/0!
7/2017 4.00NNNNNNNNNNNNNNNNYYYNNN 4 YYY19/12/20134YYNNYNNN99.99!
97SUBHASRI 13699806G21VELLORE, TN 2.0001/05/201501/0!
7/2017 2.00NNNNNNNNNNNNNNNNYYYNNN 4 NNN YNYNNNN22.00!
98MONISH 15551863G11THENI, TN 1.0001/12/201601/0!
3/2017 1.00NNNNNNNYNNNNNNNNYNNNNN 6 YYY23/02/20174YYNYNNNN48.00!
99D PRIYA BORA 7822514G29ASSAM 1.0001/06/201601/0!
6/2017 1.00NNNNNNNYNNNNNNNNNNYNNN 6 YYY 4 YNYNNNN56.00!
100KIRITHIKA 15448813G21KARUR, TN 1.0001/06/201601/0!
6/2017 1.00NNNNNNNNNNNNNNNNNNNNN 0 NNN YNNYNNN58.00!
101JABINA 7741309G21TANJAVUR, TN 1.0001/12/201601/0!
6/2017 1.00NNNNNNNYNNNNNNNNYYYNNN 6 NNN YNYNNNN12.00!
102SAI TEJASWINI 11439503G22CHITTOOR, AP 1.0001/03/201601/0!
6/2017 1.00NNNNNNNYNNNNNNNNNNNNN 4 YYY03/05/20164YYNYYNNN30.00!
103H RAJ 15527992G11KANYAKUMARI, TN 1.0601/10/201601/0!
6/2017 1.00NNNNNNNNNNNNNNNNYYYNNN 4 NNN YNYYNNN46.00!
104HAJA NAJIBUDEEN 11569130G11NAGAPATTINAM, TN 0.0301/07/201705/0!
7/2017 0.03NNNNNNNYNNNNNNNYYYYNNN 9 YYN YNYYNNN46.00!
105NEHA KESHRI 16266891G24JHARKHAND 2.0001/05/201501/0!
4/2017 2.00NNNNNNNYNNNNNNNNYNYNNN 8 YYY17/07/20154YYNYYNNN14.00!
106KANIMOXHI 13267834G21KANCHEEPURAM, TN 4.0001/02/201301/0!
2/2017 2.00NNNNNNNNNNNNNNNNNNNYNNN 2 NNN YNNNNNN10.00!
107JHELAM PRABIR 15045270F29PUNE, MH 6.0001/10/201101/0!
5/2017 6.00NNNYNNNYNNNNNNNNNYYYNNN 12 NNN YNYYNNN71.00!
108VIGASINI 10711445F21ERODE, TN 4.0001/06/201302/0!
5/2017 4.00NNNNNNNNNNNNNNNNNNNNN 0 YNY04/11/20132YYNYNNNN 6.00!
109VISHNUBARDHAN 12190733G12KADAPA, AP 2.0001/08/201523/0!
6/2017 2.00NNNYNNNYNNNNNNNNNYYYNNN 12 NNN YNYNNNN71.00!
110VISHNUVARDHAN 13991498F12CHITTOOR, AP 1.0026/03/201601/0!
2/2017 1.00NNNNNNNNNNNNNNNNNYYYNNN 4 NNN YNYNNNN68.00!
111GIRISAA 13193003G21NAMAKKAL, TN 3.0001/05/201401/0!
7/2017 3.00NNNNNNNNNNNNNNNNNNYNNN 2 NNN YNYNNNN10.00!

112JAHNAVE 14235586G22SRIKAKULAM, AP 3.0025/10/201401/0!
6/2017 2.00NNNNNNNYNNNNNNNNYYYNNN 8 YYY23/06/20174YYNYYNNN52.00!
113ANGEL HANS 8858652G24RANCHI, JHARKHAND 0.0601/05/201715/0!
5/2017 0.06NNNNNNNYNNNNNNNNYYYNNN 8 YYN YNYYNNN99.99!
114MAHALAKSHMI 1985081F21VELLORE, TN 0.0601/03/201701/0!
5/2017 0.06NNNNNNNNNNNNNNNNNNNN 0 NNN YNNNNNN 4.00!
115ASHRAFY ALAM 15946023G28BANGLADESH 0.0301/07/201715/0!
7/2017 0.03NNNNNNNNNNNNNNNNNNNN 0 NNN YNNNNNN37.00!
116AMAN MAYK 11608801G14RANCHI, JHARKHAND 2.0001/05/201515/0!
3/2017 2.00NNNNNNNYNNNNYNNYNNNN 7 YYY17/06/20164YYNYYNNN 7.00!
117SHRAVANTHI 12278705G21KANYAKUMARI, TN 2.0001/05/201501/0!
7/2017 2.00NNNNNNNYNNNNNNNYYYNNN 8 YYY30/07/20154YYNYYNNN63.00!
118NAHDA 15404276G16KANNUR, KERALA 4.0001/02/201322/0!
7/2017 4.00NNNNNNNYNNNNNNNNNNNN 4 YNY15/02/20133YYNNYNNN 6.00!
119GOPAL KUMAR 14263975G15NAWADHA, BIHAR 2.0012/07/201510/0!
5/2017 2.00NNNNNNNYNNNNNNYYYYNNN 9 YYY27/07/20174YYNYNNNN99.99!
120SONALI TOPPO 14743861G24LOHARDAGA, JHARKHAND 1.0003/12/201601/0!
3/2017 1.00NNNNNNNNNNNNNNNYYYNNN 4 NNN YNYNNNN41.00!
121K NEHA 11999020F22GUNTUR, AP 1.0001/06/201605/0!
7/2017 1.00NNNNNNNNNNNNNNNNNNNN 0 YNY28/04/20164YYNNYNNN40.00!
122UZMA ALAM 8703658G24SINGHBHUM, JHARKHAND 1.0031/10/201601/0!
4/2017 1.00NNNNNNNYNNNNYNNYYYNNN 9 YYY10/10/20164YYNYYNNN99.99!
123ULAVALA RAGHA SREE 16637391G22EAST GODAVARI, AP 1.0030/07/201606/0!
5/2017 1.00YNNNNNNYNNNNNNNNYYYNNN 6 YYY16/02/20175YYNYYNNN42.00!
124MOHNISH KUMAR 15551863G11THENI, TN 0.0901/03/201715/0!
6/2017 0.09NNNNNNNNNNNNNNNNNNNN 2 YNY23/02/20174YYNYYNNN32.00!
125NIDHAA 10751360F26MALAPPURAM, KERALA 3.0005/05/201406/0!
7/2017 3.00NNNNNNNYNNNNNNNNNNNN 4 YYY26/10/20152NYNNNNNN27.00!
126HASINI 12196106G22WEST GODAVARI, 2.0005/05/201502/0!
7/2017 2.00NNNNNNNNNNNNNNNNNNYNNN 2 NNN YNNNNNN31.00!
127APPU 16300849G11VELLORE, TN 2.0001/10/201502/0!
6/2017 2.00NNNNNNNYNNNNNNNNYYYNNN 8 NNN YNYNNNN34.00!
128JS NISHA 16307489G22KADAPA, AP 2.0001/06/201521/0!
4/2017 2.00NNNNNNNNNNNNNNNNNYNNNN 2 YNY07/02/20173YYNYYNNN30.00!
129CATHRINE 13971702D21VELLORE, TN 6.0001/11/201121/0!
4/2017 6.00NNNNNNNYNNNNNNNNNNYNNN 6 YNY10/10/20142YYNNNNNN 1.00!
130PRATHYUSHA 13633882G22KURNOOL, AP 1.0015/09/201617/0!
2/2017 1.00NNNNNNNNNNNNNNNNNNNNN 0 NNN NNNNNYN26.00!
131AKASH KUMAR 14409146G14RANCHI, JHARKHAND 1.0012/02/201614/0!
7/2017 1.00NNNNNNNNNNNNNNNYYNYNNN 5 NNN YNNNNYN99.99!
132HASRIKA 6612725G21KARUR, TN 1.0001/05/201601/0!
6/2017 1.00NNNNNNNNNNNNNNNNNNNNN 0 NNN YNNNNNN 0.00!
133HASIMA KHATUN 18747417F23HOOGHLY, WB 4.0003/11/201328/0!
7/2017 4.00YNNYNNNNNNNNYNNYYYNNN 11 YYY05/12/20134YYNNYNNN99.99!
134ALEKHYA 15670247G22EAST GODAVARI, AP 1.0031/08/201628/0!
5/2017 1.00NNNNNNNNNNNNYNNNNNNNN 1 NNN YNNYNNN 2.00!
135RIDAY UTSAB 15855841G18BANGLADESH 3.0001/06/200128/0!
4/2017 1.00YNNNNNNYNNNNNNNNNNNNN 6 YYY24/04/20174YYNYYNNN 7.00!
136SIMRAN KUMARI 15634052G25PATNA, BIHAR 1.0003/07/201604/0!
4/2017 1.00NNNNNNNNNNNNNNNNNNNNN 0 NNN YNNNNNN 6.00!
137LALCHHA 10925371G29MIZORAM 1.0001/06/201601/0!
7/2017 1.00NNNNNNNNNNNNNNNNNNNNN 0 NNN YNYNNNN99.99!
138ANDRIYA JUDSON 15847907F26ERNAKULAM, KERALA 3.0001/04/201411/0!
5/2017 3.00NNNNNNNYNNNNNNNNYYYNNN 8 YYY29/04/20143YYNNYNNN99.99!
139ABINAYA 10267841G21PUTHUKOTTAI, TN 2.0001/03/201501/0!
6/2017 2.00NNNNNNNNNNNNNYNNNNNNNN 1 YNY19/09/20153NYNYNNYN38.00!
140SATHIKA SREE 9412441G21COIMBATORE, TN 1.0027/01/201603/0!
3/2017 1.00NNNYNNNYNNNNNNNNNYYYNNN 12 YYY07/08/20173YYNYNNNN50.00!
141MD AHMED RAZA 15143330G13KOLKATA, WB 2.0021/05/201528/0!
7/2017 2.00NNNNNNNYNNNNNNNNYYNNNN 6 NNN YNNNNNN21.00!
142NANDHINI 8855712F21VELLORE, TN 3.0002/05/201405/0!
5/2017 3.00NNNNNNNYNNNNYNNYYNNNN 7 NNN YNNNNNN16.00!
143KAVIYA SREE 15628873G21COIMBATORE, TN 1.0001/06/201612/0!
4/2017 1.00NNNNNNNNNNNNNNNNNNNNN 0 YNY01/07/20164NYNYYNNN23.00!
144SADIA ISLAM 15349098G28BANGLADESH 5.0001/01/201217/0!
3/2017 2.00NNNNNNNNNNNNNNNNNNNNN 0 NNN YNNNNNN 8.00!
145ARTHI 13397291F21VELLORE, TN 4.0001/05/201301/0!
6/2017 4.00NNNNNNNYNNNNNNNNYYNNNN 6 YNY03/05/20134YYNNNNYN13.00!
146SNEHAA 16464830F21CUDDALORE, TN 3.0020/06/201418/0!
8/2017 3.00NNNNNNNNNNNNNYNNNNYNNN 3 NNN YNNNNNN 1.00!

147FAUZIA 16999513F24RANCHI, JHARKHAND 1.0025/05/201615/0!
5/2017 1.00NNNNNNNNNNNNNNNYYYNNN 4 YYN YNNNNNN73.00!
148PRIYANKA DASS 15457640G22HYDERABAD, TL 1.0001/03/201618/0!
8/2017 1.00NNNNNNNYNNNNNNNNNYNNN 6 YYY01/04/20163YYNNYNNN29.00!
149SOREN 11096549G23HOWRAH, WB 2.0031/10/201519/0!
8/2017 2.00NNNNNNNNNNNNYNYNNYNNN 4 NNN YNYNNNN 1.00!
150HARI PRIYA 17034688G22GUNTUR, AP 3.0025/04/201418/0!
8/2017 3.00NNNNNNNYNNNNYNNYYYNNN 9 YYY18/08/20153YYNNYNNY65.00!